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OFF-REFUGE INVESTIGATIONS SUB-ACTIVITY

Final Report

AK-Contaminant Exposure and Habitat Use by Declining Species of Sea Ducks Wintering Near Industrial Developments on the Alaska Peninsula and Aleutian Islands

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EXECUTIVE SUMMARY

Declines of Steller's eiders (*Polysticta stelleri*) (STEIs) have resulted in listing the North American breeding population as *Threatened* under the Endangered Species Act (Federal Register 1997; 62 CFR 31748). Exposure to hydrocarbons from boat harbors have negatively affected STEI in Norway and may be cause for concern in Alaska. Wintering STEI reside in ice-free bays near islands in the eastern Aleutians and Alaska Peninsula. Many of these harbors have substantial maritime traffic associated with commerce or fishing, that may subsequently cause elevated levels of polycyclic aromatic hydrocarbons (PAHs) in local habitats. Moreover, observations indicate that STEI roost or feed near discharge plumes from fish-processing plants and sewage treatment facilities at Dutch Harbor, Unalaska Island, and similarly at Sand Point, Popof Island. Steller's eiders may be exposed to contaminants related to these facilities.

Here, our objectives were to determine possible effects of PAH exposure to wintering STEI. We also studied Harlequin ducks (*Histronicus histronicus*; HADU) as a possible surrogate for the listed STEI. We measured 1) induction of the cytochrome P450 IA (P450) enzyme in STEI and HADU in response to PAH exposure; 2) concentration of polychlorinated biphenyl (PCB) congeners in plasma of both species and 3) contaminants in habitats used by STEI and HADU. We also studied blood parameters as indicators of health in these species related to contaminant exposure; examined patterns of habitat use and foraging locations; and evaluated P450 induction in captive STEI to aid in interpretation of data from wild seaducks.

In winter 2002 and 2003, 77 STEI and 88 HADU were captured at Dutch Harbor and Sand Point. Blood and liver samples were collected for P450 and contaminants' analyses, and the birds were released. In 2004, 15 captive STEI were dosed with a known P450 IA inducer, beta-napthaflavone (BNF). A small liver biopsy was removed from each bird via laparoscopy and analyzed for P450 induction using ethoxy-resorufin O deethylase (EROD) activity. EROD activity for STEI collected at Unalaska and Popof/Unga Islands ranged from 9.5 – 53.6 pmol/min/mg protein. Notably, the lowest values were in STEI from Captains Bay (Unalaska) and Coal Harbor (Unga Island), two sites chosen as potential reference sites. Average P450 induction was greater in HADU than STEI. Captive STEI dosed with BNF displayed definitive induction of P450. EROD activity in the control group was significantly lower than activity in either of the two treatment groups.

PCB congeners were measured in bird plasma because certain congeners can induce the P450 IA gene, thus potentially confounding the results of PAH exposure. Mean concentrations of ∑ PCB congeners in bird plasma differed by sites but not by species or sex. Concentrations of ∑ PCB congeners were highest in sea ducks collected from Unalaska Island's New Harbor and Airport Road sites. Coplanar PCB congener profiles showed little variability among species, but site specific patterns were evident, thus suggesting different sources.

Total PAHs and aliphatic hydrocarbons in blue mussels were significantly higher at the Airport Road and New Harbor sites than most other sites. Concentrations of ∑ PAHs in other potential prey of STEI were magnitudes lower than those detected in blue mussels, and organochlorines were rarely detected in any of the prey samples.

Correlations between environmental levels of ∑ PCBs in sediments or semi permeable membrane devices (SPMDs) and average ∑ PCB congeners in avian blood plasma indicated no relationship. Multiple regression analysis was used to determine the influence of ∑ PAHs in mussels, sediments, and SPMDs or ∑ PCB congeners in plasma on STEI and HADU P450 induction. Our data indicated that ∑ PAHs in mussels were best (and positively) correlated to P450 induction in STEI and HADU, whereas ∑ PCB congeners in plasma were not correlated.

In general, blood chemistry parameters for STEI and HADU differed significantly by species, and gender, and in captive vs. wild STEI. Most notably, certain liver and tissue enzymes were elevated in wild birds when compared to captive birds or literature values for closely related species.

This study determined that environmental PAH levels at some sites (ca 2600 - 3500 ppb) at Unalaska Island approached those found at superfund sites in California and Washington (ca 4100 - 7500 ppb). Induction of P450 occurred in two species of seaducks wintering in habitats proximate to industrial activity. Induction of P450 from hydrocarbon exposure was demonstrated in captive STEI. Our data indicate that recent PCB exposure as measured by blood plasma concentration is not correlated with P450 induction response of STEI and HADU. Blood chemistry parameters indicated that the general health of wild STEI and HADU may be compromised, thereby compounding possible effects of exposure to elevated levels of contaminants and other harmful factors.

Home ranges of STEI and HADU wintering in and around Dutch Harbor were larger than expected based on previous studies of HADU and seaducks, other than STEI. Mean home range

size for STEI was 37.9 ha ± 7.8 SE and 30.03 ha ± 5.2 SE for HADU. This is the first study documenting a measured home-range size of wintering STEI. Birds from both species were highly mobile within the day and between 'zones' in the Dutch Harbor area. Examination of home ranges indicated that over ½ of the marked birds utilized areas associated with sewage treatment facilities and seafood processing plant outfall pipes.

Both STEI and HADU spend relatively little time foraging compared with these same species in other northern latitude locations. We speculate that eutrophication positively affects invertebrate abundance and the wintering sea ducks were able to meet their daily energy needs with less foraging time.

In summary, STEI and HADU in industrial areas near Dutch Harbor and Sand Point appear to be exposed to contaminants which cause induction of the cytochrome P450 IA gene. Certain anthropogenic compounds, including PAHs and certain PCB congeners can cause this biochemical response in vertebrates. Plasma PCB concentrations were not correlated with P450 induction, whereas concentrations in prey items (blue mussel) of seaducks were significantly correlated with EROD activity. Moreover, most birds wintering in bays around Dutch Harbor had home ranges that overlapped outfall pipes associated with seafood processing and sewage waste, thus enhancing the exposure opportunity of these birds to contaminants.

INTRODUCTION

Steller's eiders (*Polysticta stelleri*) are listed as threatened because of declines in North American as well as global populations (Fox *et al*. 1997, Flint *et al*. 2000, Zydelis 2000). Life history studies of Steller's eiders (STEI) should aid in determining reasons for the decline (Fox and Mitchell 1997, Flint and Herzog 1999, Laubhan *et al*. 1999, Flint *et al*. 2000). Few studies have addressed the role of contaminants in this decline (Fox *et al*. 1997, Solovieva *et al*. 1998). Contaminants often are suspected in wildlife declines, but in situ studies rarely result in plausible correlations or cause and effect. This was true until recently for the effects of polycyclic aromatic hydrocarbons (PAHs) on avian species.

Polycyclic aromatic hydrocarbons are produced from the combustion or physical degradation of petroleum-based compounds. Lipophilic PAHs are persistent in the environment and have known carcinogenicity to mammals, but until recently effects on avian species have been elusive (Lee and Grant 1981). Trust *et al*. (2000) and Custer *et al*. (2000) documented induction of cytochrome P450 in wild birds from exposures to PAHs, and subsequent associated somatic chromosomal damage (Custer *et al*. 2000) or reduced productivity (Trust *et al*. 2000).

The affinity of wintering STEI for ice-free bays provided a unique opportunity to correlate contaminants to effects on wildlife. Fox *et al*. (1997) reported impacts on STEI suspected from hydrocarbon exposure at boat harbors in Norway, which is cause for concern in Alaska. Alaskan harbors or bays between the eastern Aleutian Islands and Cook Inlet shelter large numbers of wintering STEI (Larned 2000). Many of these harbors have substantial maritime traffic associated with commerce or fishing, and subsequently a potential for elevated levels of PAHs.

Fish-processing plants discharge wastewater into some Alaskan harbors, possibly confounding problems of petroleum spillage. A large proportion of the discharge is lipid-based, organic material from discarded fish matter that can account for as much as 25 % of the total fish weight (Nair 1990). The discharge can be high in nitrogenous compounds that increase biochemical oxygen demand and subsequent eutrophication (e.g., Nair 1990, Battistoni et al. 1992); thus, concerns have focused more on degraded water quality and not specifically the composition of discharge. More recently, interactions between eutrophication and cycling of contaminants received attention because of the affinity of contaminants to organic carbon (Dachs et al. 1995, Persson et al. 2000, Gunnarsson et al. 2000, Skei et al. 2000). Still, these studies did

not address the potential for bioaccumulated organic contaminants in harvested fishes, and discharge of these contaminants in lipid-based waste.

Observations by biologists indicated that STEI roost or feed near discharge plumes from fish-processing plants at Dutch Harbor, Alaska, located within Unalaska Bay on Unalaska Island. Similar observations were made at Sand Point on Popof Island further to the east. We surmised that organic contaminants may be concentrated in this waste, compounding incidences of discharged PAHs, and thus further impact marine systems that include STEI.

Objectives

Our objectives were to determine potential effects of PAHs on STEI. However, because of the listed status of STEI, we elected to include Harlequin ducks (*Histronicus histronicus*) in the study as a possible surrogate for STEI. Harlequin ducks (HADU) utilize habitat proximate to STEI but usually forage closer to shore. In this study we examined:

1) The biochemical response (induction of the enzyme P450) to hydrocarbons in STEI and HADU that roost and forage in or near boat harbors or discharged wastewater from fish processing plants.
    a) PCB coplanar congeners also were examined in STEI and HADU blood plasma as a confounding factor to interpretation of P450 induction.
    b) Also, in order to facilitate interpretation of P450 induction in wild STEI, captive STEI were dosed with a hydrocarbon standard.
2) The potential for elevated concentrations of contaminants in the habitat of STEI, including:
    a) Organochlorines, aliphatic hydrocarbons (AHs), and PAHs in common prey of STEI.
    b) PAHs and AHs in the organic flocculi (fluffy mass or tuft of organic material) that can be associated with wastewater from fish-processing plants.
    c) PAH and PCB accumulation in sediments and semi-permeable membrane devices (SPMD).
3) Measures of health indices (blood parameters) by sites, species, and gender as an indicator of avian health and potential effects of environmental exposure to contaminants.

4) Patterns of habitat use and foraging locations in relation to discharged wastewater from fish processing and harbor developments. (NOTE: Results of Obj. 4 in Appendix A).

Steller's eiders are listed and could not be sacrificed, but a minute liver biopsy could be extracted without harming the individual. The volume of liver or blood sample was insufficient for analyses of both P450 and contaminants; therefore, PCB congeners (inducer of P450 and thus a confounding factor) were determined in STEI and HADU blood plasma and organochlorine and PAH contaminants were determined in the habitat and then correlated with P450 determinations in STEI and HADU.

Blue mussels (*Mytilus* sp.) and gastropods have been identified as common components of the diets of adult STEI, with gastropods as the most representative (Bustnes et al. 2000). Polycyclic aromatic hydrocarbons are suggested to accumulate in organisms such as mollusks with poorly developed mixed function oxygenase capability; however, some studies have indicated that episodic occurrences of PAHs were short-lived in blue mussels (Miles and Roster 1999). We collected blue mussels as representative prey of STEI because these were common at most locations; other subtidal organisms (small mollusks or crustaceans) were collected when common. Aliphatic hydrocarbons were also analyzed in blue mussels to aid clarification of the source of PAHs.

In order to examine PAHs concentrations in habitat media, we collected organic flocculi (when available) that was associated with fish-processing discharge, and also sediments and water (using SPMDs). The SPMDs were used to absorb organic lipids from the water column and then analyzed for PAHs. Finally, blood samples from STEI and HADU also were collected and analyzed for health indices to determine the association of these parameters to PAH exposure.

METHODS

Study Area and Field Procedures – STEI and HADU

The study was conducted at Dutch Harbor (Unalaska Island) and Sand Point (Popof-Unga Islands), Alaska (Figure 1) in winter 2002 and 2003. Unalaska Island is in the eastern Aleutian Islands, and Popof and Unga Islands are closely adjacent islands in the Shumigan Island group near the southeastern tip of the Alaska Peninsula. Four sites were sampled at Dutch Harbor and two at Sand Point. Specific sites at Unalaska Island were Airport Road (included a seafood-

processing plant outfall), New Harbor (the site of a planned boat harbor), Captains Bay that had no processor outfalls and little shipping traffic, and Nateekin Bay across from the industrialism of Dutch Harbor. Sites at the Shumagin Islands included a seafood-processing plant outfall area (Sand Point, Popof Island) and a reference site (Coal Harbor on Unga Island). Sites were selected based on observations of habitat use by Steller's eiders (Larned 2000).

Steller's eiders and Harlequin ducks were captured using floating mist nets and decoys. Inflatable skiffs were used to drive flocks toward the nets. Capture efforts were based on a logistically reasonable time period (usually 1 – 3 days) per site in winter 2002 and 2003. Captured seaducks were transported a short distance to a mobile field laboratory where they were banded, examined for general condition, and the weight, sex, and age (adult or immature) recorded.

A wildlife veterinarian extracted a liver biopsy (approximately 0.05 g) according to established procedure (Appendix A) from STEI and HADU deemed healthy. The liver samples were immediately frozen, stored, and later transported in a liquid nitrogen container. A 4 - 5 ml sample of blood was drawn via jugular venipuncture (only if specimen exhibited good post-operative condition), transferred into heparin tubes for plasma and non-heparin tubes for serum; within 15 minutes, plasma samples (serum samples within 60 minutes) were centrifuged at 2500 rpm for 10 minutes. The resulting plasma was divided into cryovials for PCB or health parameters analyses, frozen, and transported in liquid nitrogen. All samples were transferred to a –80°C ultra cold freezer until processing. Blood smears also were prepared on slides.

Liver and Blood Analyses

Analyses of liver samples for induction of cytochrome P450 were conducted at the Wilson Laboratory of the Department of Avian Sciences, University of California, Davis. Cytochrome P450 response was measured using ethoxyresorufin-o-deethylase activity (EROD) in samples of about 4 mg wet weight. For quality assurance and control, embryonated mallard (*Anas platyrhynchos*) duck eggs were injected with 2mg/egg of beta-napthoflavone (BNF), a known P450 inducer, and their livers assayed 24 hours later as positive controls.

Liver samples were prepared for analyses by separation of hepatic microsomes by differential centrifugation. Microsome preparations were prepared from livers homogenized in 0.1 M NaPO4 buffer pH 7.4, spun down at 100,000 x g for 1 hour, and resuspended (ml/g tissue)

in 50 mM Tris containing 1 mM EDTA, 1 mM DTT, 20% v/v glycerol, at pH 7.4. EROD activity was measured as described by (Trust et al. 2000) according to the method of Bruke and Mayer (1974) as adapted to a fluorescence microwell plate scanner (Melancon 1996). Modifications were determined in triplicate in a 96 well plate at 25°C using a Packard FluoroCount microplate fluorometer (Packard Instrument Company, Meriden, CT). Each well contained 1 ul of microsomes, 159 ul of 2.5 uM final concentration 7-ethoxyresorufin in 50 mM Tris-buffer pH 8.0. The addition of 40 ul of 1.34 mM final concentration of NADPH initiated activity. Fluorescence was measured at excitation/emission wavelengths of 530 and 590 nm, respectively, at 1-minute intervals for 6 minutes. EROD was expressed as picomoles per minute per milligram of protein (pmol/min/mg protein). Protein was determined using the Bradford reagent (Sigma, St. Louis, MO).

Blood plasma was analyzed for: 1) Enzymes – Alkaline phosophatase (ALP), aspartate aminotransferase (AST), creatine phosphokinase (CK or CPK), lactate dehydrogenase (LDH), and amylase; 2) Proteins – albumin, total protein, globulin, and albumin/globulin (A/G) ratio; 3) Lipids – cholesterol; 4) Sugars – glucose; 5) Minerals – calcium and phosphorus; 6) Electrolytes – bicarbonate, chloride, potassium, and sodium; and 7) Waste Products – uric acid and bile acids. Blood smears were analyzed for avian complete blood count (CBC), which included estimates of average white blood count (WBC), heterophils, lymphocytes, eosinophils, and basophils, and a qualitative assessment of thrombocytes, polychromasia, and parasites. Blood samples were analyzed by IDEXX Veterinarian Diagnostic Laboratory, Sacramento, CA.

Habitat Sampling and Analyses

When and where possible, 3 flocculi and 3 composite samples of the most common molluscan or crustacean species were collected in the shallow subtidal or intertidal habitat from each of the 6 sites in mid-winter 2002, using SCUBA or by hand. Flocculi samples in the water column or from the benthos were collected with hand-held nylon nets and invertebrates by net or by hand. A new set of clean nets were used at each site. Exact species collected was determined by the most common found at the sites. Samples were stored in the field in sterile polypropylene bags. Each sample consisted of approximately 15 grams (blotted wet weight) of flocculi or soft tissue of invertebrates; invertebrate composites comprised about 20 mussels or crustaceans, or 30 – 50 gastropods. Soft tissues were extracted within 1 – 2 hours using stainless steel instruments

cleaned between samples with hexane and then acetone, and then rinsed with distilled H_2O. These tissues were stored in chemically clean jars, and frozen until analyses.

Sediment and SPMD samples were collected at six sites. Locations were chosen because they were within known STEI foraging areas (< 10 m depth), and they were coincident with prey and flocculi samples previously collected. Up to 250 grams of sediment was collected using chemically-clean Eckman or Ponar dredges. Dredge material was collected several times from a location and dumped collectively into a clean stainless steel. Large rocks and debris was removed and sample was thoroughly mixed with a stainless steel spoon. ICHEM®, Series 300 jars were filled with sediment sample and jars were frozen at -4^0 C as soon as possible after collection.

Standard SPMDs and associated deployment hardware were obtained from Environmental Sampling Technologies (EST), St Joseph, Missouri. Each standard SPMD consisted of a 91.4x2.54 cm (36x1 inch) low-density polyethylene tube filled with one milliliter of high purity (98%) triolein. One SPMDs was required for each site in order to attain an analytical detection limit in the parts per billion (ppb) range for PAHs. Care was taken to ensure that SPMDs are not inadvertently contaminated by the deployment and retrieval activities of project personnel. In order to minimize the amount of exposure time to airborne contaminants during deployment operations, each SPMD was mounted on a separate standard "spider" deployment rack by the manufacturer. The deployment racks remained in airtight containers until immediately before in-water deployment. At deployment, each "spider" rack was placed into a standard 16.5x15 cm (6.5x6 inch) stainless steel deployment canister and submerged as quickly as possible. Air exposure time and specific deployment location, time, date and approximate deployment depth was recorded for each SPMD.

In order to ensure that the analytical results only reflect water-borne PAH concentrations, the confounding effects of air-borne PAHs inadvertently sampled during deployment and retrieval operations were minimized by the use of a trip blank. One trip blank was used for the study and was exposed to air during both the deployment and retrieval operations of one device. The trip blank was be re-sealed in its canister following sample SPMD submersion and then frozen for storage.

Approximately 28 calendar days after deployment, the SPMDs were recovered, removed from their deployment devices and placed in sealed canisters. The canisters were frozen as soon as possible and shipped to EST for extraction and analysis. All SPMDs (sample devices, replicates and trip blank) underwent sample clean-up and recovery procedures at EST using standard SPMD methodology (McCarthy and Gale 1999; www.spmds.com).

Flocculi and invertebrate samples were analyzed for PAHs, AHs, and organochlorines (OCs), including PCB congeners, at Mississippi State University Chemical Laboratory, State College, Mississippi. Sediment and SPMD samples were analyzed for all analytes mentioned above, except for AHs.

Sample specific detection limits for PCB congeners averaged 0.27 ng/g (range: 0.01 – 8.47 ng/g) across all samples analyzed. Samples specific detection limits averaged 3.5 ng/g (range: 3.1 – 5.0 ng/g) for PAHs and were less variable than PCB congeners. Limits of detection (LOD) for all aliphatic hydrocarbons and chlorinated pesticides averaged 10 ng/g and 2 ng/g, respectively. Analytical accuracy and precision was assessed using spiked sample recovery and duplicate analyses on 5 % of all samples. Spiked recoveries for PCB congeners averaged 99% (range: 54- 126%), 96% (range: 16 – 121%) for PAHs, 84% (range: 67 – 97%) for chlorinated pesticides, and 82% (range: 63 – 102%) for aliphatic hydrocarbons. Relative percent difference averaged 1.4% (range: 0 – 30.5%) for PAHs, 0% (range: 0 – 0%) for chlorinated pesticides. Aliphatic hydrocarbons had lower precision (x = 32.7, range: 0 – 123%), largely because duplicate samples for several compounds were below the LOD, and therefore did not have any significance to the results.

Dosing Study

In order to facilitate interpretation of field results, 15 STEI from Dutch Harbor were captured in March 2003 and flown to the Alaska Sealife Center (ASLC) in Seward, Alaska. These birds were held in quarantine for 9 months and determined by Center veterinarians to be in good condition and health prior to dosing.

In January 2004, captive STEI were separated by males and females and then randomly assigned to one of three groups; assignment of treatments to these groups was also random. Birds in one group were exposed by orally administered gelatin capsule containing BNF at 20 mg/ and another group at 100 mg/ kg body weight. Dose levels were established according to Renauld et

al. (1999) and Melancon (personal communication) in order to prevent harm to the captive STEI. The third group was control and birds received an empty gelatin capsule to experience similar handling stress. Birds were fasted for approximately 12 hours prior to the initial dosing. A single oral dose was administered every 24 hours for two consecutive days after the initial dosing. The birds were provided food and water *ad libitum*, as they had been provided throughout quarantine.

At the end of three days of dosing (on the fourth day of the study), each bird underwent surgery and a liver biopsy extracted and placed into liquid nitrogen. Birds were allowed to recover in isolation for approximately 2 hours prior before being returned to their pens. After surgeries, birds were no longer needed for experimental purposes and were retained by ASLC as part of their exhibit flock.

Statistical Analyses

Total (∑) PCB congeners were quantified in waterfowl blood plasma, sediments, and SPMDs. Total PAHs were quantified in all samples except seaduck blood plasmas. Organochlorines were quantified in blue mussels, crustaceans, the gastropod *Tegula*, and flocculi**.** Aliphatic hydrocarbons were analyzed only in blue mussels. All data were log-transformed prior to analyses. We used a 3-factor ANOVA to test the effects of species, site, and gender on ∑ PCB (interactions were not significant so main effects model was used). Correlation of biochemical measures to contaminants in habitat was conducted using Pearson Correlation coefficients and 95 % confidence intervals (CI).

We examined possible effects of PAHs in mussels, sediment, or SPMDs on EROD induction in STEI and HADU while controlling for effects of PCBs in duck blood plasma. We assumed environmental ∑ PAHs (in SPMDs, sediments, or blue mussels) were reflective of PAHs in seaducks. We first regressed average PAHs against average PCBs and then regressed the residuals against average EROD activity against residuals from regressions. This analysis is comparable to a multiple regression but allows for graphical representation of the PCB controlled effect of PAHs on EROD. We also examined possible effects of PCBs in duck blood plasma on EROD while controlling for effects of ∑ PAHs in mussels. Multiple regression also was used to test effect of ∑ PCB congeners in plasma, species, and island on EROD activity in seaducks (islands were modeled instead of site to facilitate simpler analysis on log transformed data (analogous to ANCOVA).

Blood chemistries were averaged by each species and site. Years were pooled since values were not expected to be different within years. Average ranges for blood values for other duck species (e.g., adult male mallard, white-winged wood duck [*Cairina scutulata*], American black duck [*Anas rubripes*], and Peking duck [*Anas domesticus*]) were taken from primarily laboratory studies and compared with STEI and HADU blood values (Beynon et al. 1996; Fairbrother et al. 1990; Franson 1982; Fowler and Miller 2003; Johnson-Delaney and Harrison 1996; Mulley 1979; Shave 1986; Spano et al. 1987).

We used MANOVA to test for overall differences in blood values among species, sites, and gender. Blood values were log-transformed, and then a full-effects MANOVA (including all interactions) was performed to determine if any differences in blood indices were consistent among treatments. All interactions were not significant (< 0.78, F = 1.29, P < 0.20) thus only main effects of treatments (sites, species, or gender) were modeled. Univariate ANOVA was then used to determine which particular blood values differed among treatments followed by post-hoc Tukey Kramer mean separation tests to determine differences among groups. Blood enzymes, electrolytes, and other indices should be interpreted in conjunction with one another to help detect trends that may indicate individual health issues, therefore correlation matrices were used to examine relations among blood values and associations with EROD and PCB values for each species.

Mean reported values are back-transformed geometric means for contaminants and arithmetic means for blood indices.

RESULTS and DISCUSSION

During winter 2002 and 2003, 38 STEI and 69 HADU were captured at Dutch Harbor and 39 STEI and 19 HADU captured at Sand Point, biopsies and other tissues extracted, and these analyzed for biochemical response to hydrocarbons and PCB exposure (Table 1). In 2004, 15 captive STEI were dosed with BNF and a biopsy of liver tissue removed for P450 analysis.

Avian Biochemical Response and Contaminant Blood Chemistries

EROD and Free-Ranging Seaducks

Both STEI and HADU demonstrated biochemical response to exposure by hepatic induction of P450 (Figure 2). EROD activities for STEI collected at Unalaska and Popof/Unga

Islands were similar for 2002 and 2003; the mean ranged from 9.5 – 53.6 pmol/min/mg protein. Notably, the lowest values were in STEI from Captains Bay (Unalaska) and Coal Harbor (Unga Island). Average EROD activity was significantly greater in HADU than STEI ($F= 113.0$, $P < 0.00001$, $df_{1,133}$); the mean ranged from 46.1 – 280.4 pmol/min/mg protein, with highest levels reported in HADU from both Nateekin Bay and Sand Point ($F = 8.13$, $P < 0.00001$, $df_{4,133}$). Mean EROD activity was consistently higher in HADU from 2003 than 2002 and in STEI at two of three comparable sites. Levels of EROD in individual ducks indicated that activity generally was greater in HADU but extrinsic factors could induce relatively high levels of EROD activity in individuals of STEI. Levels of EROD activity ranged from 4.6 - 412 pmol/min/mg protein in individual STEI and 20 - 688 pmol/min/mg protein in HADU.

PCB Congeners in Plasma

Mean concentrations of ∑ PCB congeners in bird plasma differed by sites ($F = 4.7$, $P = 0.001$ $df_{4,133}$) but not by species ($X_{STEI} = 11.5$ ng/g; $X_{HADU} = 12.9$ ng/g; $F = 0.00$, $P = 0.99$, $df_{1,133}$) or sex ($X_{MALE} = 13.8$ ng/g; $X_{FEMALE} = 9.8$ ng/g; $F = 2.6$, $P = 0.11$, $df_{1,133}$) (Figure 3). Concentrations of ∑ PCB congeners were highest in sea ducks collected from Unalaska Island's New Harbor (X = 16.7 ng/g) and Airport Road (X = 14.7 ng/g) sites but lowest concentrations were detected at Nateekin Bay (X = 7.7 ng/g), similar to that at Sand Point (X = 9.8 ng/g) on Popof Island

Plasma PCB congener concentrations in STEI and HADU were not consistently higher in one species than the other across sites (Figure 4). Coplanar PCB congener profiles showed little variability among species but site specific patterns were evident suggesting different sources (Figure 5). Relatively high proportions of PCBs 118 (11 – 13%), and 138 and 153 (14 – 23%) characterized profiles from STEI and HADU from Airport Road, New Harbor, and Nateekin Bay, Unalaska Island Congener profiles from Captains Bay had slightly lower concentrations of these congeners but higher proportions of lower chlorinated congeners such as PCBs 52, 15, and 11. Also, interspecific differences were more apparent; PCB 11 comprised 17% for HADU and 0% for STEI, while PCB 138 comprised 25% for STEI but only 9% for HADU. In notable contrast, profiles for both STEI and HADU from Sand Point at Popof Island had high proportions (17 – 22%) of the lower chlorinated congener PCBs 11 and 15. Only STEI were

collected at Coal Harbor (Unga Island); the highest proportion was PCB 138 (32 %), followed by PCBs 52 and 202 (14% each).

Contaminants in Prey and Media

Blue Mussels

Blue mussels were obtained from all sites except Nateekin Bay. Mean concentrations of ∑ PAHs in these mussels were significantly higher at the Airport Road (x = 2634 ng/g) and New Harbor (x = 3487 ng/g) sites at Unalaska, than all other sites (Figure 6). These ∑ PAH concentrations were comparable to those reported in blue mussels from highly polluted sites, e.g., EPA superfund sites Lauritzen Canal (4100 ng/g) at San Francisco Bay and Eagle Harbor (5224 – 7498 ng/g), Puget Sound, Washington (Miles and Roster 1999, Krishnakumar 1994). Concentrations of ∑ PAHs were virtually undetected at Captains Bay (Unalaska Island) and Coal Harbor (Unga Island), indicating these sites sufficed as references sites for lower PAH exposure than all other sites.

Total PAHs and aliphatics in blue mussels were highly correlated ($R^2 = 0.73$, $P < 0.0001$). Patterns of mean ∑ AHs in blue mussels was similar to that of ∑ PAHs (e.g., highest at Airport Road [x = 5309 ng/g] and New Harbor [x = 3670 ng/g]) except that ∑ AHs in blue mussels were also low in concentrations at Sand Point similar to Captains Bay and Coal Harbor (Figure 6).

Profiles of PAHs in blue mussels were similar at Airport Road and New Harbor sites with a higher proportion of substituted or alkylated PAHs, indicating exposure of mussels to PAHs from petroleum spillage more than combustion of petroleum products (Law and Biscaya 1994) (Figure 7). Alkylated PAHs C4-napthalenes comprised 20 and 30% and C2-phenanthrenes comprised 14 and 10% of the PAHs detected at Airport Road and New Harbor; C1-phenanthrenes comprised 10% at both sites. Benzo(e)pyrene (high molecular weight and more rings and potentially more toxic) usually formed from the combustion of petroleum-based products, comprised 7 – 8% of the PAHs in these mussels. Expectedly, individual PAH constituents were detected in low frequency at the Captains Bay and Coal Harbor sites. At Captains Bay, fluoranthene, phenanthrene, and benzo(e)pyrene comprised 27%, 25%, and 18% of individual PAHs detected. These compounds are more commonly the result of combustion (or incomplete combustion) of petroleum-based products. Benzo(e)pyrene comprised the majority (44 and 55%) of PAHs at Coal Harbor and Sand Point (Figure 7).

Geographic patterns of AH composition were similar to PAH profiles (Figure 8); however, certain higher proportioned AHs indicated sources from organic origins. The AHs pristane (25 – 37 %) and phytane (11 – 19 %) comprised the majority of aliphatics at the Dutch Harbor sites, and were also notable at the Sand Point. These non-petroleum based oils occur naturally and could be elevated as a result of fish-processing. Proportional composition of AHs was more evenly distributed at the Shumagin Islands at both Sand Point and Coal Harbor; similar AH compounds comprised 5 % or more of the AHs.

Other Prey and Flocculi

Concentrations of ∑ PAHs in other potential prey of STEI were magnitudes lower than those detected in blue mussels (Figure 9). Concentrations of PAHs in these prey items did not differ among the sites where available. The gastropod *Tegula* sp. was common in sufficient abundance subtidally only at Unga/Popof Islands, with notably higher concentrations of ∑ PAHs at Sand Point in the vicinity of an outfall and the boat harbor than *Tegula* from the more remote Coal Harbor. The shrimp *Pandulus* sp. was collected subtidally at the Airport Road site, and amphipods and isopods collected in the intertidal at Captain's Bay and New Harbor; low concentrations of ∑ PAHs were similar at these three sites. Flocculi were available in sufficient quantity only at Sand Point (Popof Island) and New Harbor (Unalaska Island). No major outfall occurred at New Harbor and notably concentrations of ∑ PAHs were higher at Sand Point.

Fluoranthene, perylene, and phenanthrene comprised 5 – 30 % percent of the individual constituents of ∑ PAHs in crustaceans from Dutch Harbor (Figure 10). Pyrene comprised 45 % of ∑ PAHs in *Tegula* from Sand Point, whereas dibenz(a,h)anthracene comprised 43 % of ∑ PAHs in *Tegula* from Coal Harbor. Perylene comprised all of the ∑ PAHs in flocculi from New Harbor, whereas 16 individual PAHs comprised 1 – 12 % of the ∑ PAHs at Sand Point. Pyrene is a byproduct of the manufactured gas process and other incomplete combustion processes; perylene is thought to derive from chemical transformations within soils or sediments, although its origin is uncertain.

Organochlorines were rarely detected in any of the prey samples. For example, p,p'-DDE was detected in one blue mussel composite sample from Airport Road (3.0 ng/g) and no other chlorinated pesticides were detected. Also, ∑ PCBs were detected in only 2 blue mussel samples from Airport Road (15.0 and 120.0 ng/g). The lack of detectable PCBs in prey indicated

that either prey were very efficient at metabolizing these xenobiotics, or that PCB congener accumulation in bloods of STEI and HADU were acquired from sources in addition to prey. For example, contaminants that accumulate in the oily surface microlayer of oceanic waters (e.g., Cross et al. 1987, Hardy et al. 1987, Risnyk et al. 1987) could be transferred to seaducks via preening.

Physical Media

Only 1 – 2 samples of sediments or SPMDs were taken per site, therefore only descriptive information is provided. In sediments, ∑ PAHs were highest at Captains Bay (161 ng/g) and Coal Harbor (108 ng/g), and markedly lower at all other sites (< 31.1 ng/g; Figure 11). Total PCBs did not appear highly elevated (< 0.7 ng/g) and were uniform among sites.

In SPMDs, ∑ PAHs followed similar patterns observed in seaducks and blue mussels, i.e., highest levels at Airport Road and New Harbor (ca. 6900 ng/device) and lowest at Coal Harbor (1364 ng/device). Expectedly, ∑ PAHs were generally higher in SPMDs than mussels that can efficiently metabolize PAHs. Both SPMDs and mussels indicated high ambient levels of PAHs at Dutch Harbor. Total PCBs in SPMDs were also highest at Airport Rd (52.8 ng/device), but notably lowest at New Harbor (7.5 ng/device).

No significant correlations were found between or among ∑ PAHs and ∑ PCBs in SPMDs and sediment samples ($R^2 < 0.13$, $P \leq 0.50$). This was not surprising as surficial sediments typically do not retain high levels of volatile organic contaminants.

Correlations of Environmental and Avian Parameters

Correlations between environmental levels of ∑ PCBs in sediments and average ∑ PCB congeners in avian blood plasma indicated no relationship (STEI $R^2 < 0.16$, HADU $R^2 = 0.01$) probably because of the low levels of ∑ PCBs in sediments across sites (Figure 12). However, levels of ∑ PCBs in SPMDs and in avian blood plasma also were not correlated (STEI $R^2 = 0.10$, HADU $R^2 = 0.02$, Figure 12). Concentrations of ∑ PAHs in blue mussels were negatively correlated ($R^2 = 0.66$) with levels of ∑ PAHs in sediments (the relationship was significant at $P = 0.10$), and not correlated with levels in SPMDs ($R^2 = 0.05$, $P = 0.35$; Figure 13). Levels in sediments or SPMDs seemed poor indicators of concentrations detected in biological media.

Multiple regressions were used to determine effects of ∑ PAHs in mussels, sediments, and SPMDs (assuming these media were reflective of STEI and HADU to PAH exposure) on

STEI and HADU EROD, while controlling for the effects of ∑ PCB congeners in plasma, sediments, and SPMDs. Total PAHs in mussels were best (and positively) correlated to EROD activity in STEI ($R^2 = 0.54$, P = 0.09) and HADU ($R^2 = 0.80$, P = 0.06) than sediments or SPMDs (Figure 14). The negative correlation of ∑ PAHs in sediments and STEI EROD was unexplainable but consistent with negative correlation with ∑ PAHs in blue mussels. When controlling for effects of ∑ PAHs in mussels, ∑ PCB congeners in plasma were not correlated with EROD activity in STEI ($R^2 = 0.12$, P = 0.55) or HADU ($R^2 = 0.23$, P = 0.30). No correlation was apparent between PCB congeners in avian plasma and EROD when accounting for variations due to island or species (Figure 15).

Correlations between ∑ PAHs and EROD were unanticipated because PAHs were not measurable directly in the seaducks. However, these findings indicate that apparently high environmental PAH contamination was more instrumental in EROD induction than correlations with PCB congeners in the bloods of seaducks.

Avian Health Blood Parameters

In general, health indices differed significantly by site (□ < 0.29, F = 1.88, P < 0.0001, $df_{88, 461}$), species (□ < 0.36, F = 9.55, P < 0.0001, $df_{22, 116}$), and gender (□ < 0.75, F = 1.80, P < 0.025, $df_{22, 116}$). Health indices in general also differed significantly in captive vs. wild STEI (□ < 0.31, F = 13.7, P < 0.00001, $df_{22, 136}$). The following brief synopses of individual health indices with accompanying univariate tests are provided to further interpret MANOVA results. Stress, trauma, exposure to toxins, muscle or liver damage, infection, disease, state of satiation, state of hydration, or even rough handling can affect blood chemistries. Blood enzymes, electrolytes, and other indices often are reviewed relative to each other to aid in interpretation of meaning.

Differential Complete Blood Count

White blood cells (WBC) are the first defense against infection or trauma. White blood cell estimates for both STEI and HADUs were similar to the baseline range established for white-winged wood ducks (Beynon 1996; Figure 16), however, HADU WBC counts were higher than STEI (F = 9.32, P < 0.05, $df_{1, 139}$). Site effects were not detected, but females tended to have higher WBC estimates than males (F = 4.52, P = 0.013, $df_{1, 139}$). Estimates of WBC did not differ between wild and captive STEI.

Heterophil levels usually increase with stress, trauma, toxicosis, or neoplasia. Heterophils

are produced as a response to acute body stress, e.g., from infection, infarction, or trauma. Heterophil levels were significantly higher in Nateekin Bay than those detected in Captains Bay or New Harbor ($F = 4.10$, $P = 0.0035$, $df_{4, 139,}$ Figure 17). Heterophil levels did not differ significantly between species or gender. Heterophil levels were highly elevated over baseline studies for all groups. Lymphocyte levels often are elevated where heterophil levels are elevated and were highly correlated ($R^2 = 0.96$) and similarly, lymphocyte levels were significantly higher in ducks from Nateekin Bay than New Harbor ($F = 2.52$, $P = 0.044$, $df_{4, 139,}$; Figure 18). However, lymphocytes were within reported baseline ranges for all groups.

Eosinophils are often seen at the site of invasive parasitic infestations and allergic (immediate hypersensitivity) responses. Basophils are uncommon in avian blood slides and sometimes are associated with inflammatory responses or tissue damage. Eosinophils and basophils were not consistently quantified on the blood slides so statistics comparing differences in species, gender and sampling site were not calculated and data is presented graphically in Figures 19 and 20. Eosinophils levels were within baseline ranges for captive STEI but highly variable for wild seaducks, whereas basophil levels were within range for all groups.

Polychromasia is an indicator of regenerative anemia. Polychromasia was described as slight in all blood samples and moderate in only two STEI from New Harbor and 1 captive STEI individual. Thrombocytes, also known as platelets are one of the main components of the blood. They form clots that seal injured areas and stop bleeding. Thrombocytes were not enumerated but were described as adequate in number for all samples. No blood parasites were reported for any samples and red blood cell morphology was described as normal or that the cells do not appear toxic or reactive for all samples.

Enzymes

Alkaline phosophatase (ALP) may increase from damage to kidney, intestine, or liver, and in birds, is primarily associated with bone growth and repair. Levels of ALP in HADU were higher than those in STEI ($F = 68.15$, $P < 0.0001$, $df_{1, 139}$; Figure 21). Levels of ALP in HADU (average range 1225 – 1525 IU/L) were highly elevated over reported baseline levels, whereas levels in all STEI (average range 50 – 210 IU/L) were within range. Because ALP levels in HADU (and STEI) were similar over all sites, this may indicate a species-specific baseline under wild conditions rather than indication of physiological impairment. Levels of ALP did not differ

significantly among sites or between genders or wild vs. captive STEI.

Aspartate Aminotransferase (AST) is usually associated with liver, heart, skeletal muscle, brain and kidney tissues. Elevated levels are often caused by hepatocellular or muscle damage. Levels of AST did not differ significantly among sites or between gender, but STEI had significantly higher AST values than HADU ($F = 33.83$, $P < 0.0001$, $df_{1, 139}$). Values for AST also were significantly higher in wild than captive STEI ($F = 24.31$, $P < 0.00001$, $df_{1, 83}$; Figure 22). Notably AST levels were highly elevated above baseline levels particularly in wild STEI (compared to HADU), and within range for captive STEI.

Creatinine Kinase (CK) is an enzyme primarily associated with muscle tissue. It can be elevated after long flights or with muscle injury or trauma. Levels of CK were higher in all wild birds compared to captive STEI ($F = 7.26$, $P = 0.0085$, $df_{1, 83}$; Figure 23). Levels of CK were particularly elevated in STEI captured at Airport Road and Nateekin Bay than STEI and HADU from all other sites ($F = 4.37$, $P = 0.0023$, $df_{4, 139}$). Levels of CK did not differ significantly between species or gender. Similar to AST, CK levels were elevated above baseline levels particularly in wild STEI. Levels of CK were within range for captive STEI, and near baseline for HADU from Captains Bay and Sand Point.

Elevated lactate dehydrogenase (LDH) often is associated with liver or cardiac disease, or skeletal muscle damage. Levels of LDH were higher in birds from the Airport Road site compared to those from Sand Point ($F = 2.44$, $P = 0.049$, $df_{4, 139}$). Levels of LDH also were significantly higher in (all) wild STEI than captive STEI ($F = 21.05$, $P = 0.00002$, $df_{1, 83}$; Figure 24). Notably, LDH levels were elevated in wild seaducks in relation to reported normal ranges, and within range for captive STEI. Although LDH levels did not differ significantly between species (or genders), these levels were generally higher in wild STEI than HADU.

Amylase is an enzyme that originates in the pancreas, liver, and small intestine. Increased values of amylase are usually associated with pancreatic diseases, e.g., pancreatitis. Amylase values were significantly lower in (wild) STEI than those in HADU ($F = 13.87$, $P = 0.0003$, $df_{1, 139}$), and than those in captive STEI ($F = 9.73$, $P = 0.0025$, $df_{1, 83}$; Figure 25). Amylase did not differ significantly among sites or between genders. Amylase levels in all groups were well below baseline, indicating baselines for wild seaducks differed substantially from reported baseline values. Low amylase values may be indicative of severe liver disease (including hepatitis), conditions in which the pancreas fails to secrete enough enzyme for proper digestions

(pancreatic insufficiency), and when toxic materials build up in the blood.

Certain of the enzymes evaluated (ALP, AST, and LDH) are associated with various tissues but are usually attributed to liver damage when elevated. When considered in conjunction with CK (an enzyme primarily associated with muscle tissue damage), it can determine if enzyme elevations are due to muscle or other tissue damage. Clinically, LDH is considered a liver enzyme although it is present in most avian tissues. Levels of LDH and CK were correlated ($R^2 = 0.75$ $P < 0.0001$ for STEI; $R^2 = 0.76$, $P < 0.0001$ for HADU) indicating that increased LDH levels were probably due to muscle damage. Additionally, LDH and AST levels were correlated ($R^2 = 0.85$, $P < 0.0001$ for STEI; $R = 0.86$, $P < 0.0001$ for HADU), again indicating that increased values were due to stress or damage to muscle tissue rather than liver tissue. The enzymes ALP, AST, CK, and LDH were all elevated in wild STEI compared with captive STEI, indicating that wild birds suffer from more muscle or tissue damage than birds in captivity (Table 2).

Proteins

Total protein (TP) levels are made up of both albumen and globulin. Total protein, albumen, globulin levels as well as the ratio of albumin to globulin (A:G) are evaluated in relation to one another in order to determine specifics about avian health. For example, elevations in total protein levels can be caused by an inflammatory infection which is associated with an increase in globulin levels resulting in a decrease in the A/G ratio. Globulin is the "antibody" protein important for resisting disease. Even if the total protein levels are within normal range, a decreased A:G ratio will indicate if there is an inflammatory infection. Dehydration can cause an increase in albumen levels.

Levels of TP, albumin, and globulin levels in STEI and HADU from all sites were generally within ranges described as normal for adult male mallards and Peking ducks (Fairbrother 1990, Spano 1987) (Figures 26 – 28), but some interspecific differences were noted. Ratios of A:G ($F = 13.45$, $P = 0.0003$, $df_{1, 139}$; Figure 29) and globulin ($F = 5.47$, $P = 0.0207$, $df_{1, 139}$) were higher in HADU than those in STEI. Except for these differences, TP, albumin, globulin, and A:G ratios levels were not significantly different among sites or between species or genders, nor did levels differ between captive and wild STEI.

Lipids

Cholesterol is synthesized in the liver and elevations in cholesterol may be associated

with lipemia (presence of excess lipids on the blood) or high fat diets. Cholesterol levels were more elevated in captive vs. wild STEI, perhaps due to dietary difference ($F = 9.19$, $P = 0.003$, $df_{1, 83}$; Figure 30). Cholesterol levels were higher in ducks sampled at Airport road than those from Nateekin Bay and Captains Bay ($F = 3.19$, $P = 0.012$, $df_{4, 139}$), and in wild STEI than HADU ($F = 6.07$, $P = 0.015$, $df_{1, 139}$). Cholesterol levels did not differ between genders.

Captive STEI were fed a diet of 80-90% Mazuri Sea Duck Diet (Purina Mills, LLC, St. Louis, MI) and 10-20% krill at the Sea Life Center (Tuula Hollmen, Sea Life Center, personal communication). In the wild, principal foods in marine areas include bivalves and gastropods (Bustnes et al. 2000). The variation in diet composition may also be cause for differences in blood cholesterol, as well as levels of bile acid, glucose, and electrolytes phosphorus, sodium, and bicarbonate. Appendix 2 outlines diet composition of common food items for wild STEI and Mazuri diet.

Sugars

Glucose levels are mediated by hormones that affect carbohydrate metabolism as well as tissue metabolism and storage. Hyperglycemia can be higher transiently with stress and hyperthermia, whereas hypoglycemia can result from starvation, malnutrition, liver disease, bacterial infection, or endocrine dysfunction. Glucose levels did not differ significantly among sites or between species. Blood glucose levels were significantly higher in wild than captive STEI ($F = 13.35$, $P < 0.0004$, $df_{1, 83}$; Figure 31). Glucose levels were higher in HADU than (wild) STEI ($F = 25.62$, $P = 0.0001$, df1).

Electrolytes

Increased bicarbonate levels indicate metabolic alkalosis and decreased levels indicate metabolic acidosis. Bicarbonate levels in captive STEI were significantly lower than their wild counterparts ($F = 123.8$, $P < 0.000001$, $df_{1, 83}$; Figure 32). Bicarbonate levels did not differ significantly among sites or between species or gender.

Decreased values for chloride may indicate conditions that cause water retention. Chloride levels did not differ significantly among sites or between species, gender, or captive vs. wild STEI (Figure 33).

Sodium levels are linked to the hydration status of an animal. Decreased sodium levels may result from hydration status and increased levels may indicate increased uptake through diet,

or dehydration. Sodium levels were significantly lower in captive than wild STEI ($F = 19.82$, $P < 0.00003$, $df_{1, 83}$; Figure 34). Sodium levels did not differ significantly among sites or between species or gender.

Sodium and chloride ion levels are often interdependent, as sodium levels increase, chloride levels increase as chloride is drawn across cell walls into the blood. Sodium and chloride levels were positively correlated with one another for both STEI ($R^2 = 0.80$, $P < 0.0001$) and HADU ($R^2 = 0.74$, $P < 0.0001$).

Increased potassium levels in blood may be due to metabolic or respiratory acidosis, severe tissue damage, or dehydration. Decreased potassium levels may be an indicator of decreased diet, or loss through vomiting or diarrhea. Potassium levels were elevated in birds sampled at Nateekin Bay compared with those from Airport Road ($F = 2.47$, $P = 0.047$, df; Figure 35). Potassium levels did not differ significantly between species or gender, or sample site, nor did they differ between captive and wild STEI.

Minerals

Calcium levels in avian blood can fluctuate due to reproductive condition, and are usually elevated in egg producing females. Lipemia can also elevate blood calcium levels. Calcium levels were higher in HADU than STEI ($F = 4.91$, $P = 0.028$, $df_{1, 139}$; Figure 36), but all levels were within reported normal ranges. Calcium levels did not differ significantly among sites or between gender, or captive vs. wild STEI.

Most of the body's phosphorus is combined with calcium within the skeleton; a small proportion exists in the blood and other soft tissues and body fluids as phosphate (PO4) ions. Elevations of phosphorus levels may indicate a decrease in calcium levels or diseased liver. Phosphorus levels did not differ significantly between HADU or STEI, or gender. Phosphorus levels were higher in seaducks sampled at Sand Point than those at New Harbor ($F = 4.00$, $P = 0.0042$, $df_{4, 139}$; Figure 37). Phosphorous levels were higher in wild than in captive STEI, whose levels were within reported normal ranges ($F = 14.14$, $P = 0.0003$, $df_{1, 83}$).

Waste Products

Uric acid is the end product of protein breakdown in birds. High protein diets or over-exertion may cause elevations in uric acid and severe tissue damage or starvation causes decreased uric acid values. Uric acid levels were consistently higher in HADU than STEI (F =

5.27, P = 0.02, $df_{1, 139}$; Figure 38), and probably represented species specific differences as levels did not differ significantly among sites or between gender and were generally within reported baseline. Uric acid levels were significantly higher in captive than wild STEI (F = 4.69, P = 0.03, $df_{1, 83}$).

Bile acid levels are a good indicator of liver function since cholesterol is broken down in the liver into bile acids where they are either absorbed into the small intestine or should be cleared out of the blood by liver cells. Therefore, increased bile acids in blood are an indicator of impaired hepatic function. Bile acids can be increased with increases in food consumption. Bile acid levels were significantly lower at Sand Point than Nateekin Bay and New Harbor (F = 4.11, P = 0.0035, $df_{4, 139}$), and also lower in captive than wild STEI (F = 12.9, P = 0.0008, $df_{1, 83}$; Figure 39). Bile acid levels did not differ significantly between species or gender.

EROD Activity

Blood values were correlated with EROD activity and measured PCB values in blood. There was correlation between bile acid and EROD values in HADU (R = -0.264, P = 0.0249) and WBC count and PCB values (R = -0.34, P = 0.0039). No correlations were detected between EROD, PCB, and blood values in STEI. In general, there were no meaningful correlations detected.

EROD and Dosed Captive STEI

Baselines of P450 1A in captive STEI exposed to 20 mg/kg body weight BNF were induced 4-fold in comparison to controls, indicating a definitive biochemical response by STEI to exposure to PAHs (Figure 40). Induction at the 20 and 100 mg/kg body weight treatments were similar and significantly higher than the control group (F = 4.15, P = 0.04, $df_{2,12}$). Captive STEI exposed to 100 mg/kg body weight BNF induced on average at levels (13.6 pmol/min/mg protein) similar to STEI in the 20 mg treatment (16.2 pmol/min/mg protein). Free-ranging STEI displayed (mean) induction in excess of 50 pmol/min/mg protein, and one individual had induction as high as 412 pmol/min/mg protein. Ambient levels of PAHs at Dutch Harbor were highly elevated, suggesting that exposure was continuous and probably greater experienced at the 100 mg/kg dose, which may explain the difference.

CONCLUSIONS

This study was conducted under very harsh, winter maritime conditions in Alaska, and was one of the most comprehensive studies of its kind on a listed species under in situ conditions. Environmental PAH levels at Unalaska Island approached those found at superfund sites in California and Washington, particularly at a site of concern to the U.S. Fish & Wildlife Service, i.e., New Harbor. The highest concentrations of PAHs were found in a potential prey of STEI and an ambient measuring device (SPMD), however much lower concentrations were detected in other potential prey or sediments. Utilizing specialized surgical procedures requiring the expertise of a number of veterinarians, induction of cytochrome P450 in STEI and HADU was detected without apparent harm to the specimens. Induction was probably more in response to exposure to PAHs than PCBs. Further, induction of P450 from hydrocarbon exposure was demonstrated in captive STEI. Study of blood parameters indicated that the general health of wild seaducks may be compromised therefore compounding the potential inimical impacts from exposure to elevated levels of contaminants as well as other harmful factors, e.g., disease. Abnormal (when compare to baseline studies or captive birds) health indices such as enzymes associated with liver dysfunction were particularly notable, although correlations of these enzymes also indicated that muscle damage rather than liver damage may better explain elevation of these enzymes.

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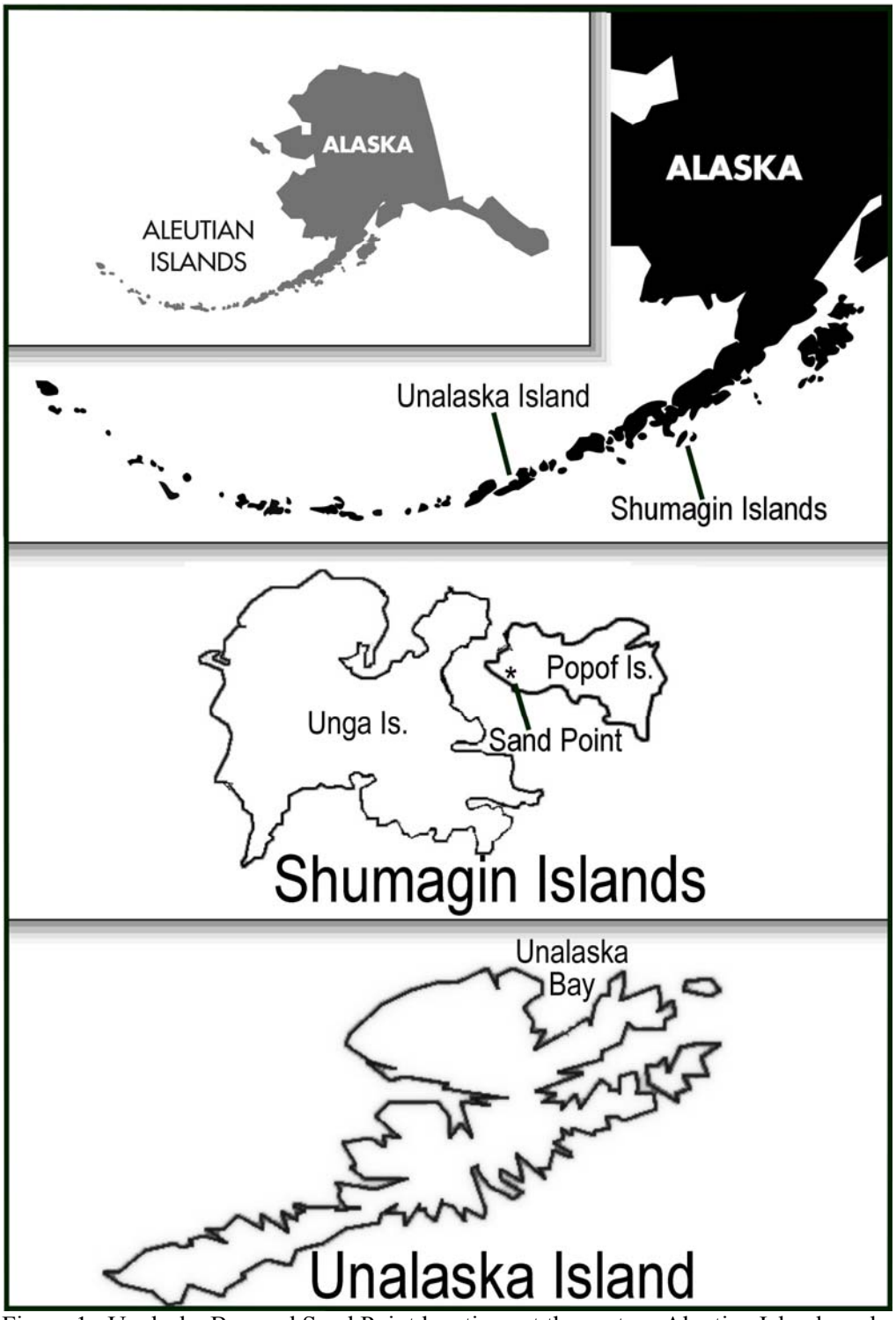
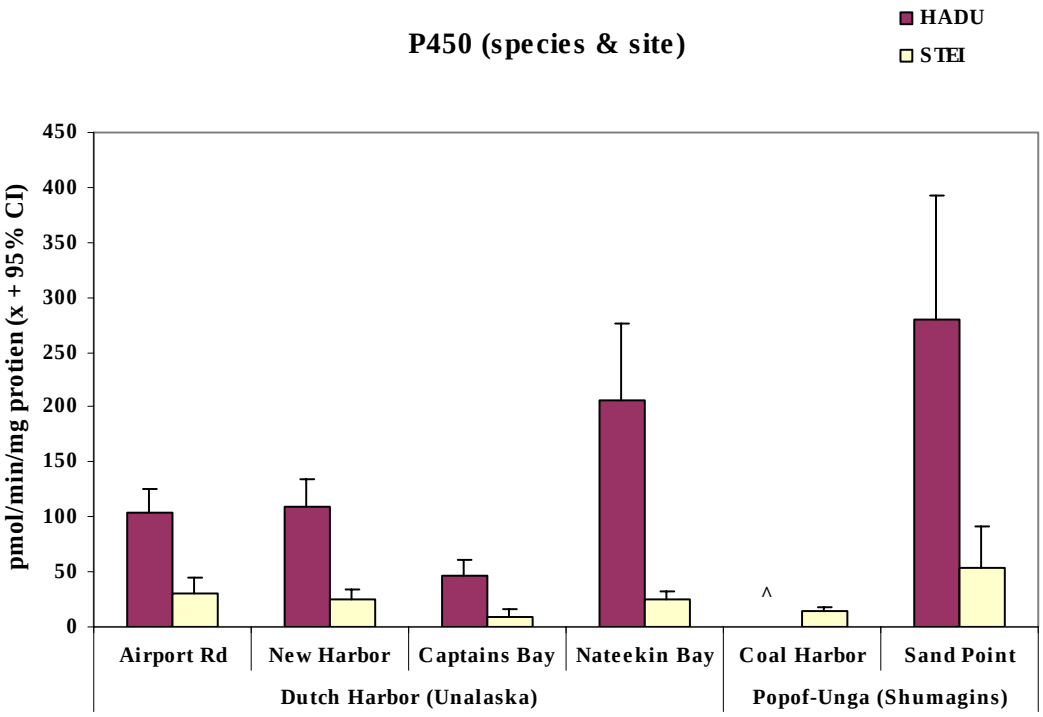


Figure 1. Unalaska Bay and Sand Point locations at the eastern Aleutian Islands and Alaska Peninsula.



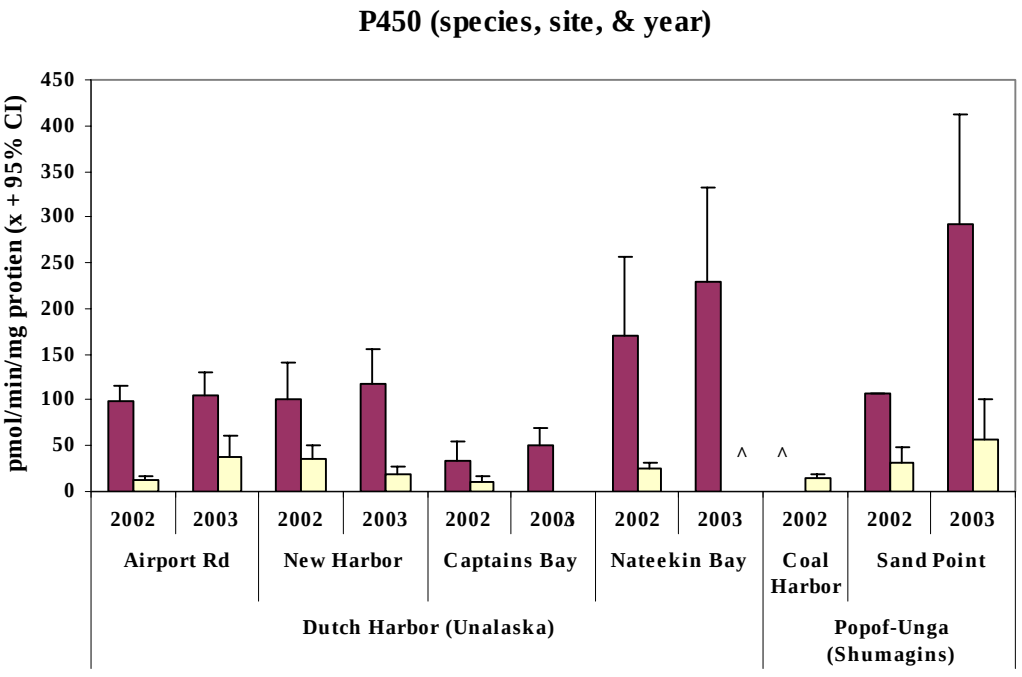


Fig 2. Mean concentrations (+ 95% CI) for hepatic P450 activity in Steller's eiders (STEI) and Harlequin ducks (HADU) collected at sites at Unalaska and the Shumagin Islands, Alaska winter. 2002-2003. Carets (^) indicate unsuccessful capture attempts for STEI or HADU.

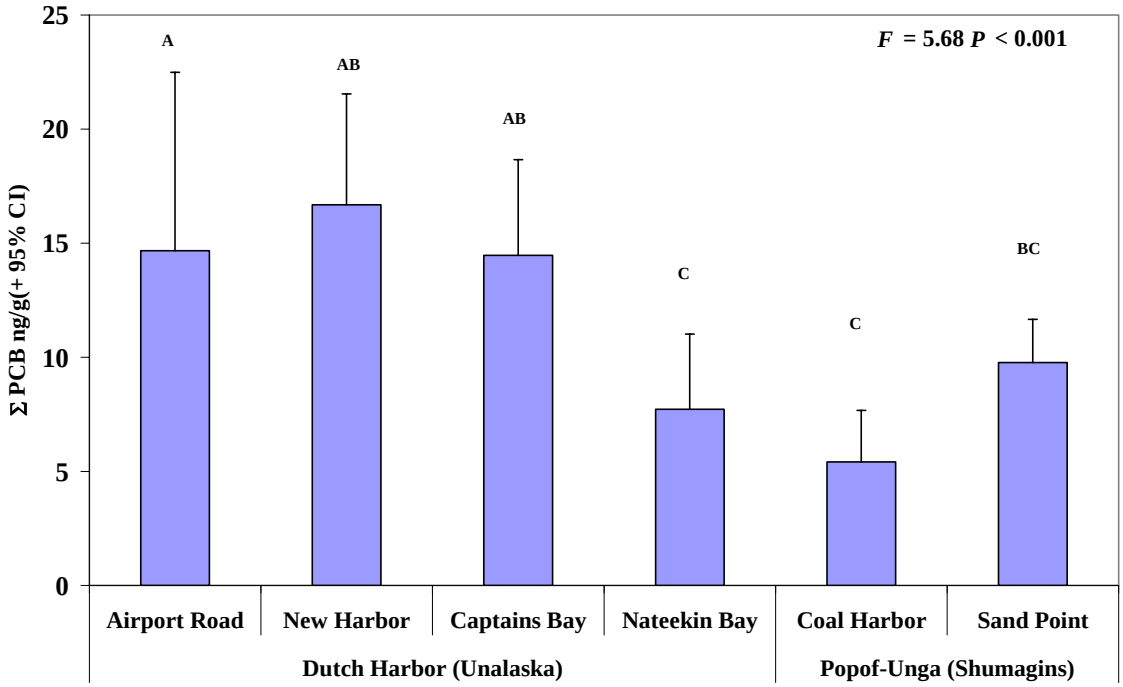


Figure 3. Geometric mean concentrations (+95% CI) for PCB congeners in blood plasma from Steller's Eiders and Harlequin Ducks sampled at sites in Unalaska and the Shumagin Islands, Alaska during 2002-2003. Sites with different letters differed significantly. Species (P = 0.99) and gender (P = 0.11) did not differ significantly; Shumagin sites were not pooled and a 1-way ANOVA was conducted. PCBS do not include congeners 126 and 180 which were not quantified for samples collected in 2002.

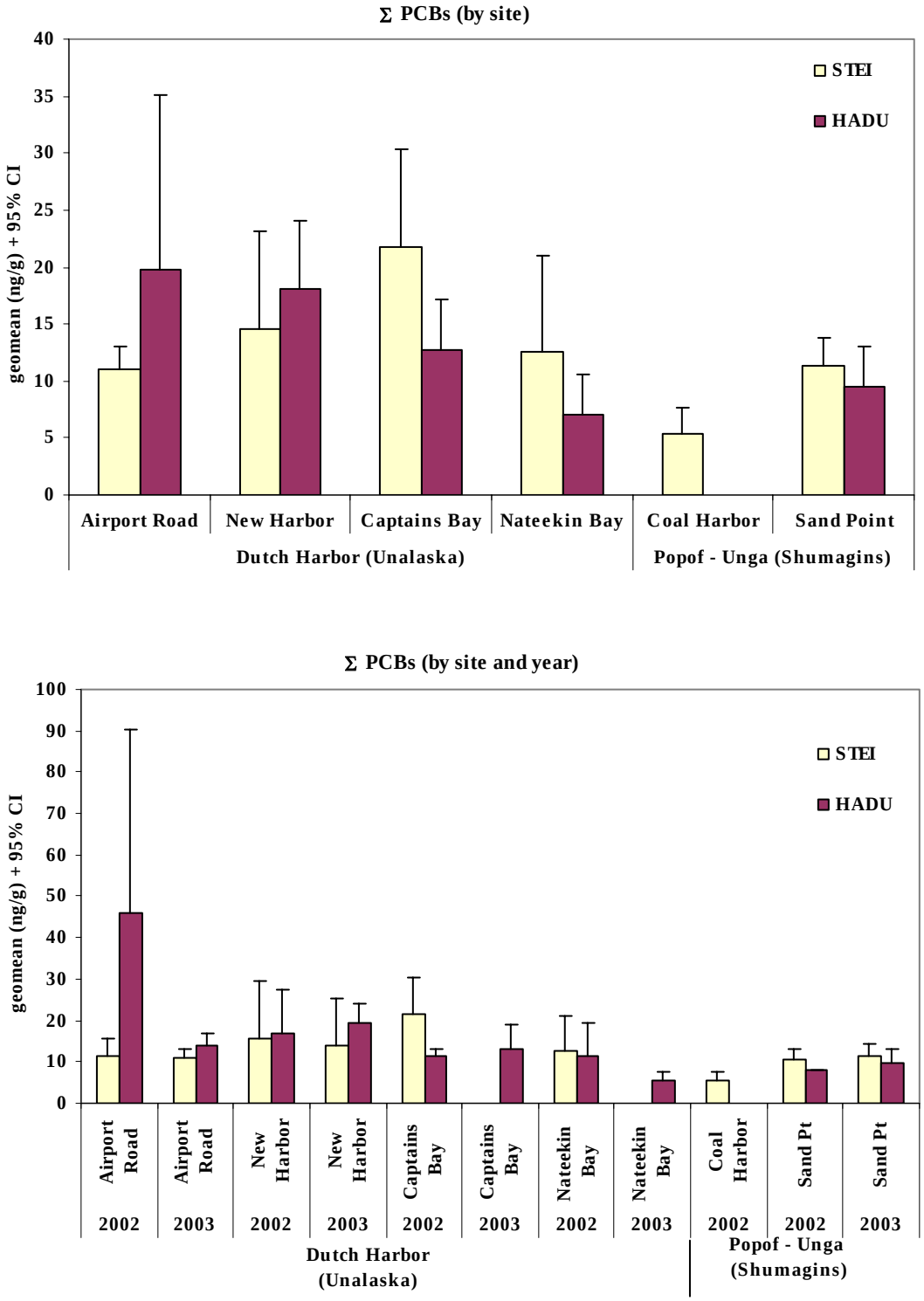


Figure 4. Geometric mean concentrations (+ 95% CI) for PCB congeners in blood plasma from Steller's eiders (STEI), Harlequin ducks (HADU) from sites in Unalaska and the Shumagin Islands, Alaska during 2002-2003. PCB congeners do not include congeners 126 and 180 which were not quantified for samples collected during 2002.

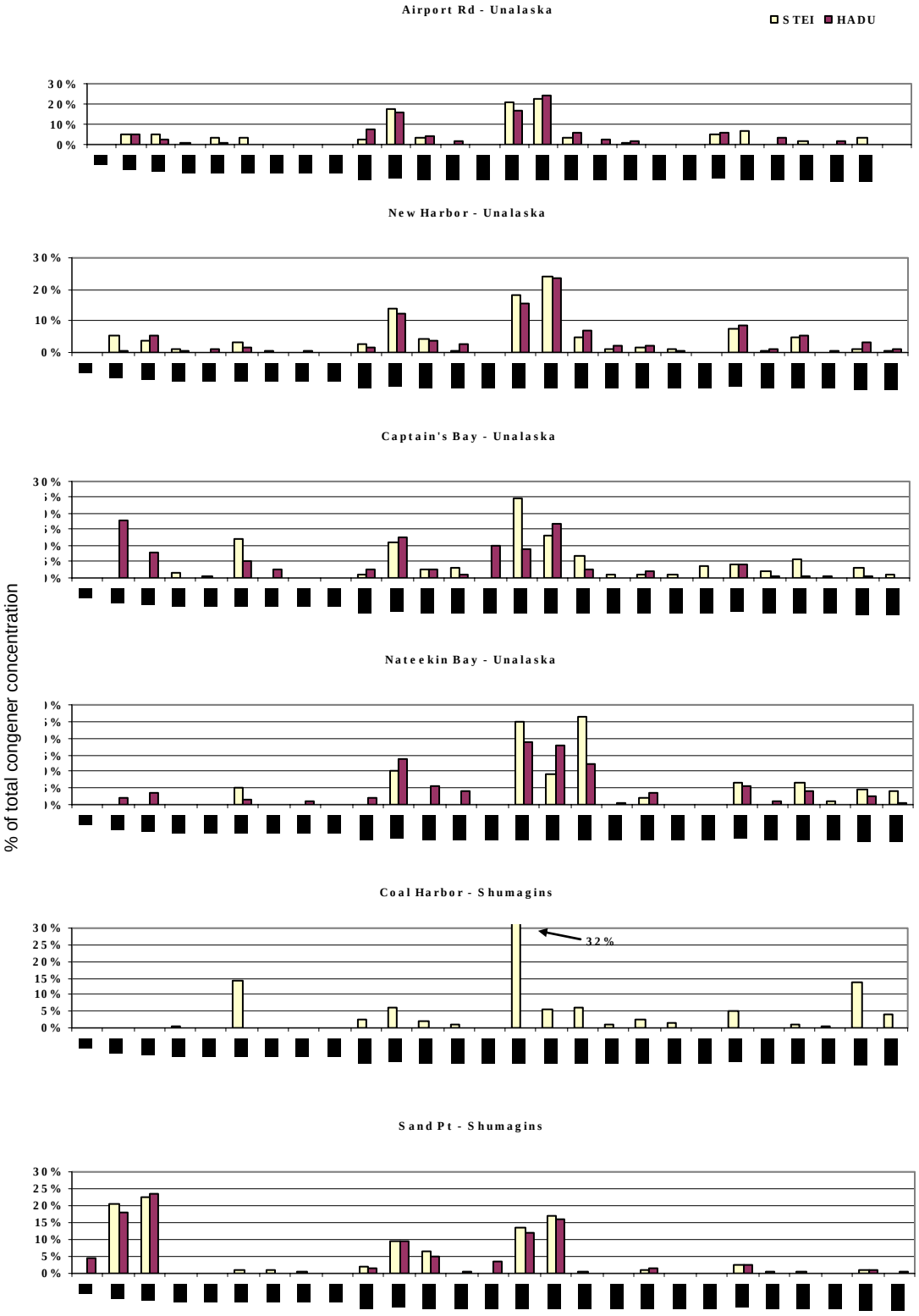


Figure 5. PCB congener profiles (wet weight) in blood plasma from Steller's eiders (STEI) and Harelquin ducks (HADU) sampled at sites in Unalaska and the Shumagin Islands, Alaska, during 2002-2003.

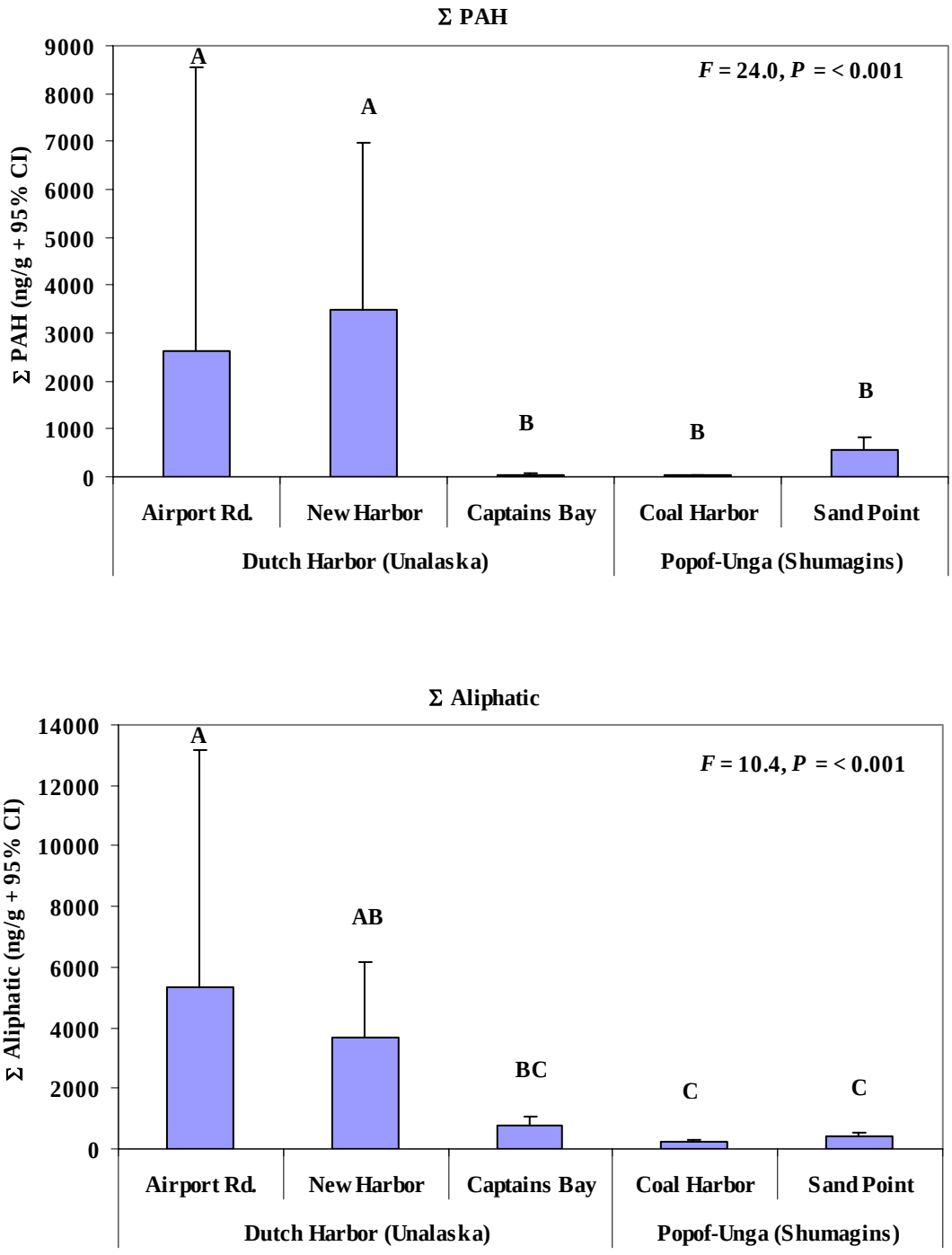


Figure 6. Geometric mean (+ 95% CI) for PAH and Aliphatic hydrocarbons in blue mussels sampled from sites at Unalaska and the Shumagins Islands, Alaska, 2002.

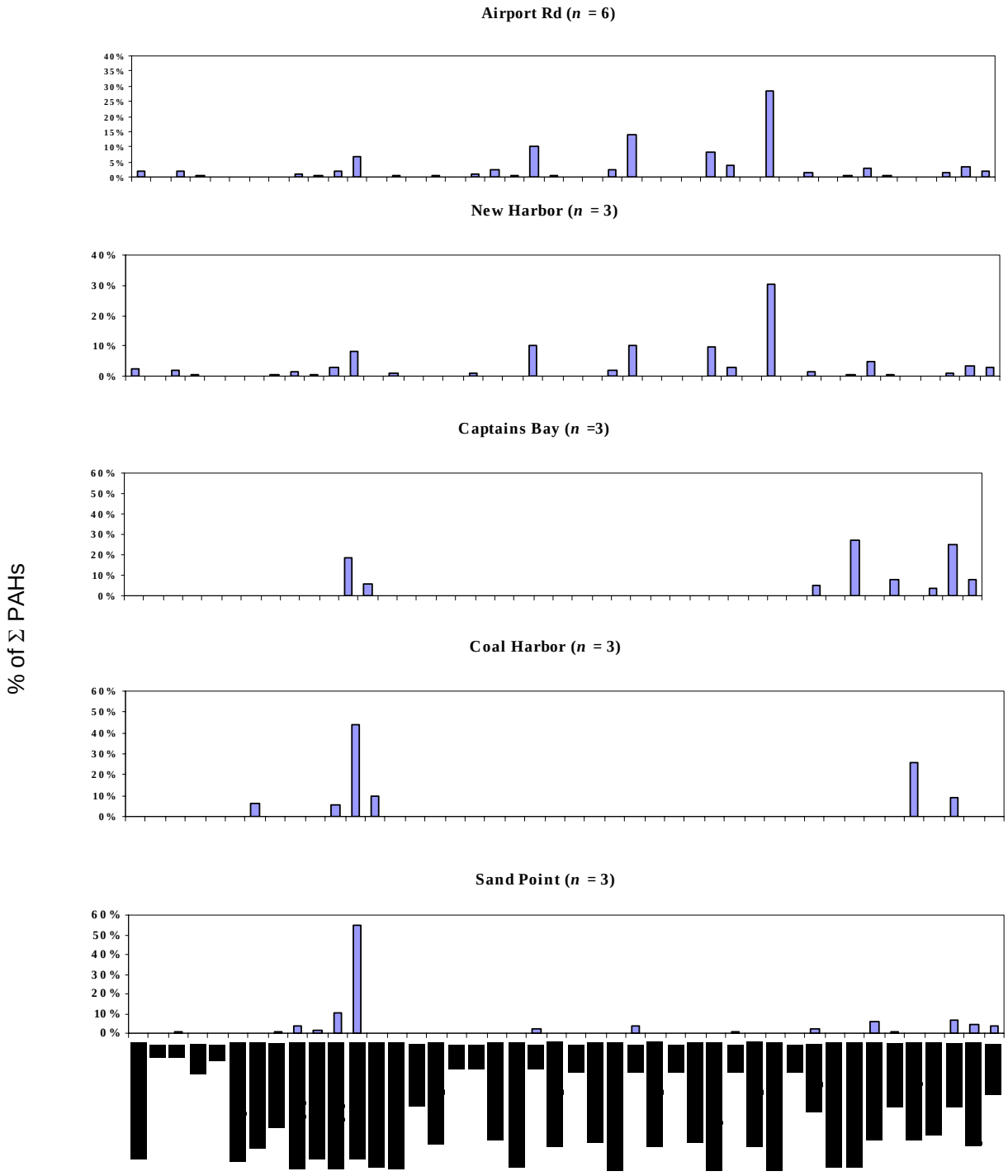


Figure 7. Percent composition (% of total PAHs, wet wt) for polycyclic aromatic hydrocarbon compounds in blue mussels sampled at sites in Unalaska and the Shumagin Islands, Alaska, during 2002-2003. Note y-axis scale varies among sites.

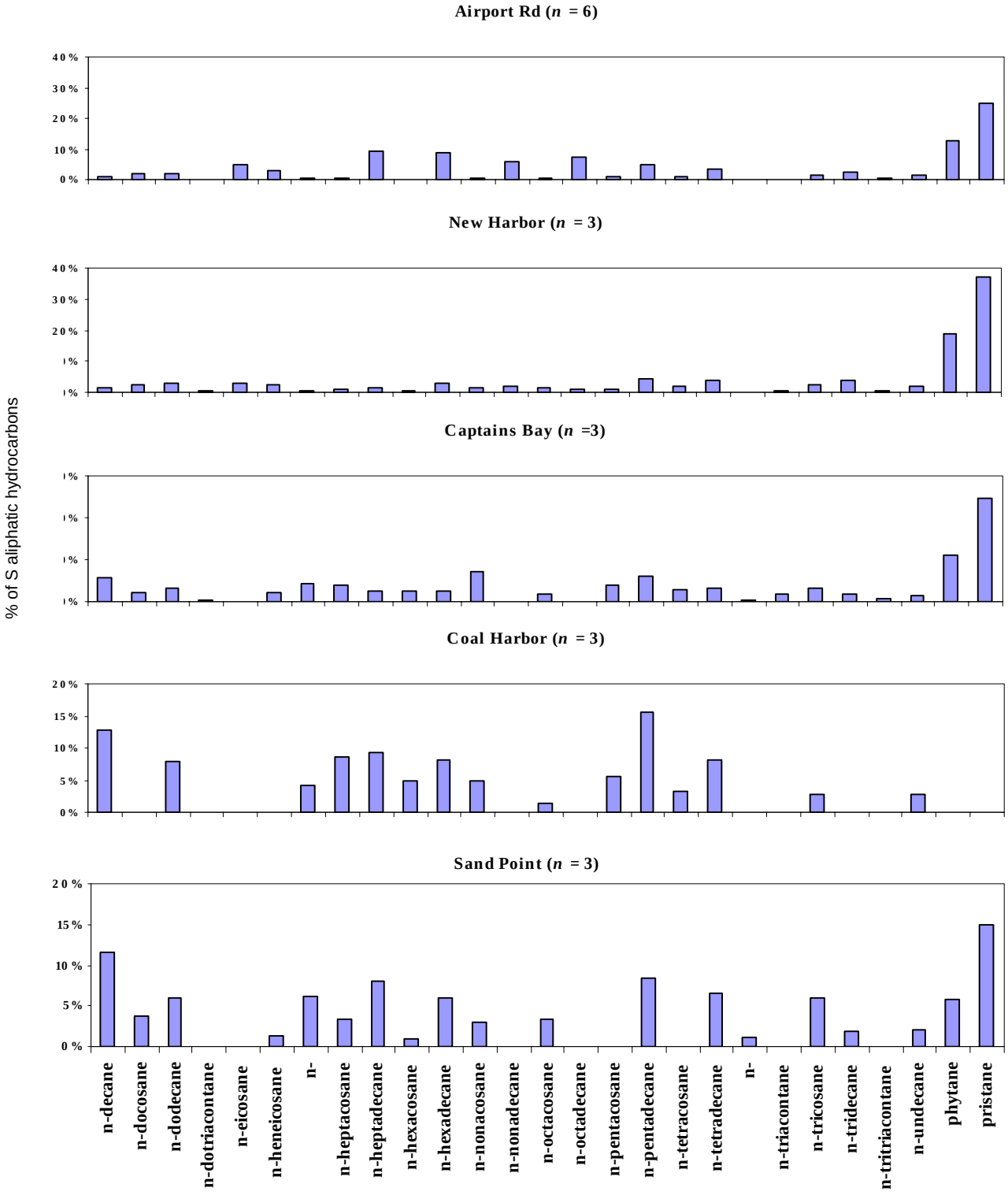


Figure 8. Percent composition (% of total, wet wt) for aliphatic hydrocarbon compounds in blue mussels sampled at sites in Unalaska and the Shumagin Islands, Alaska, during 2002-2003. Note y-axis scale varies among sites.

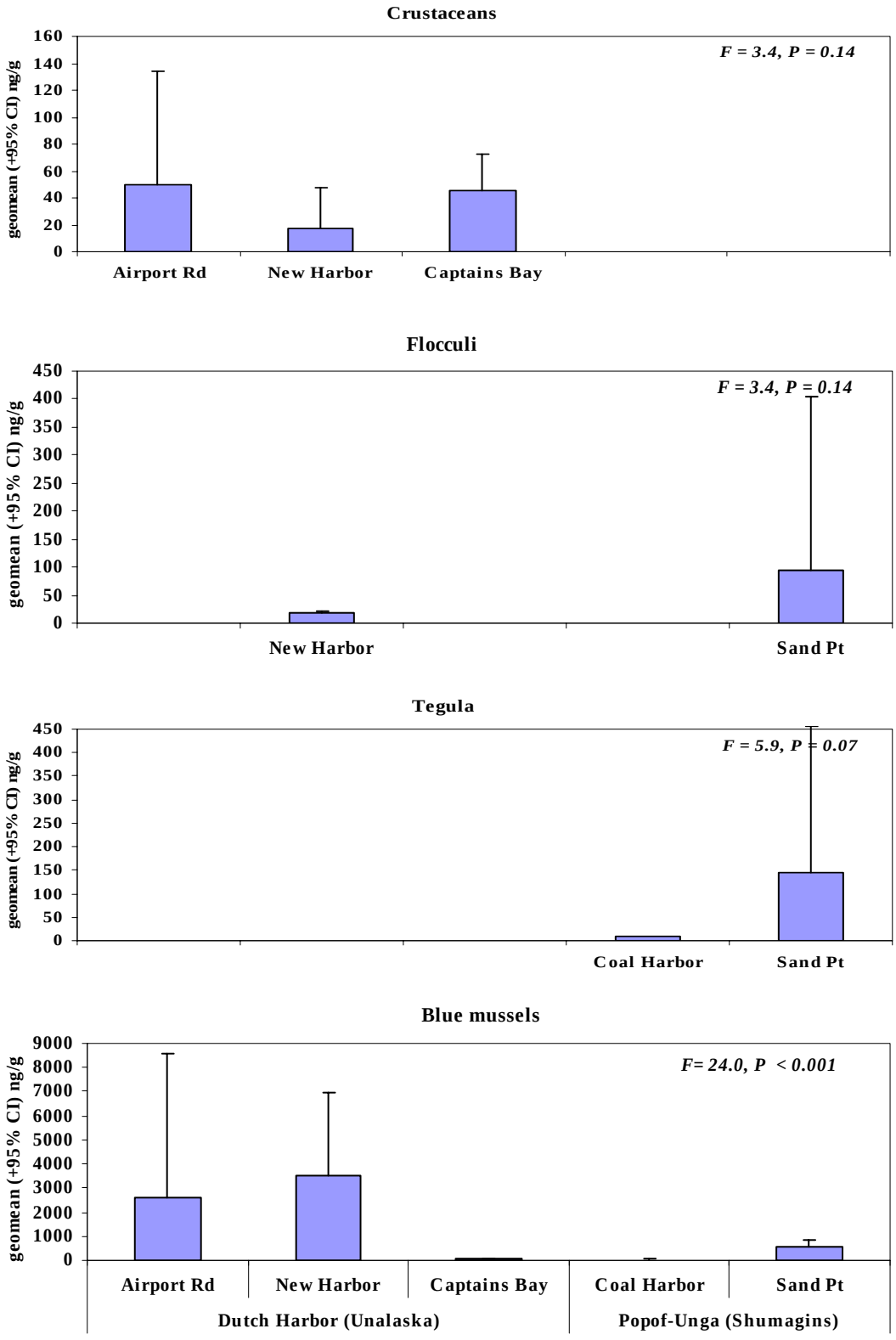


Figure 9. Geometric mean concentrations for PAH in crustaceans, Tegula, floculi, and blue mussels collected at sites at Unalaska and the Shumagin Islands, Alaska, 2002. Note y-axis varies among species. Unlabelled sites indicate no samples were collected.

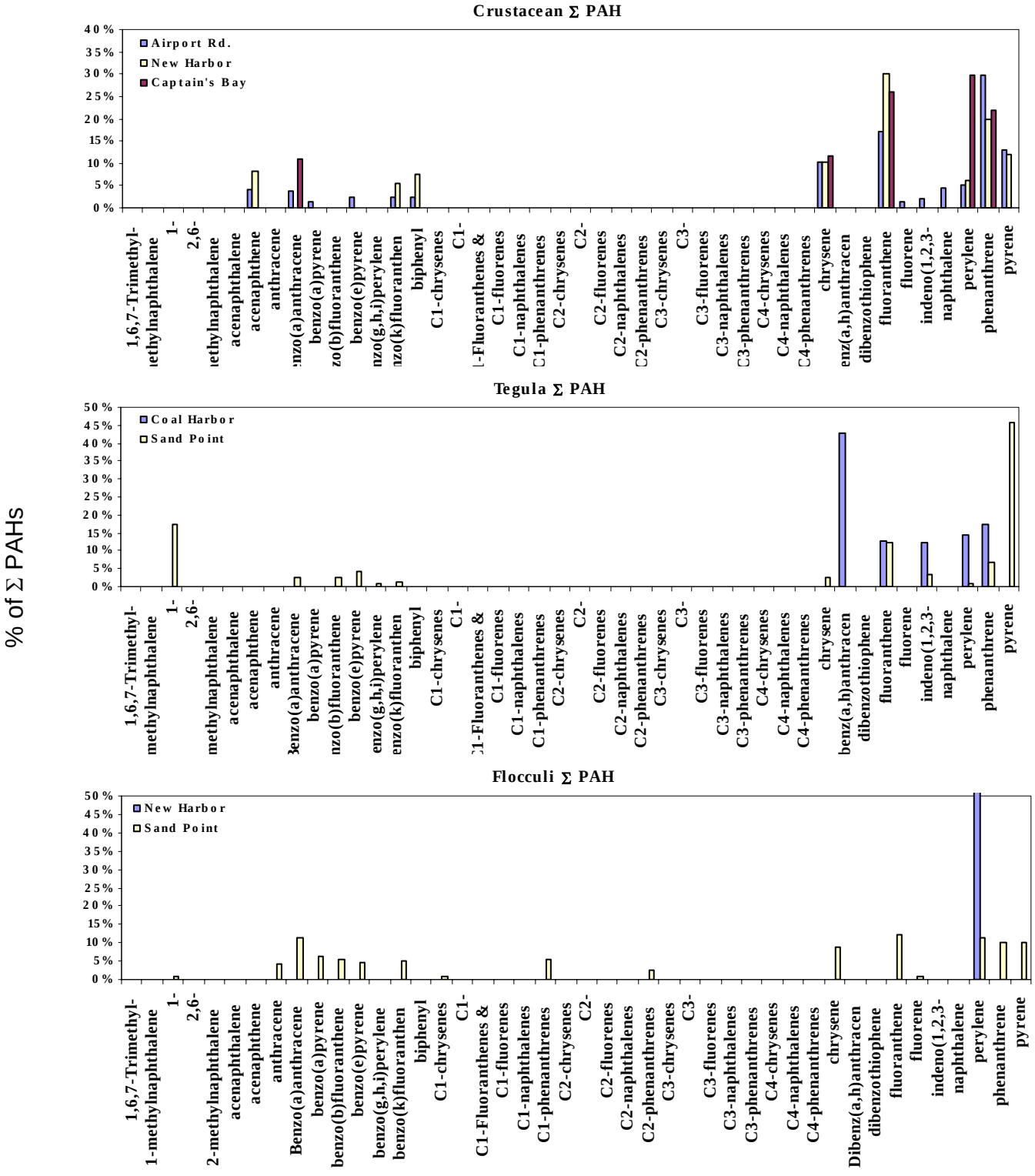


Figure 10. Percent composition (% of total PAHs, wet wt) for polycyclic aromatic hydrocarbon compounds in invertebrates and organic matter sampled at sites in Unalaska and the Shumagin Islands, Alaska, during 2002-2003. Note y-axis scale varies among sites.

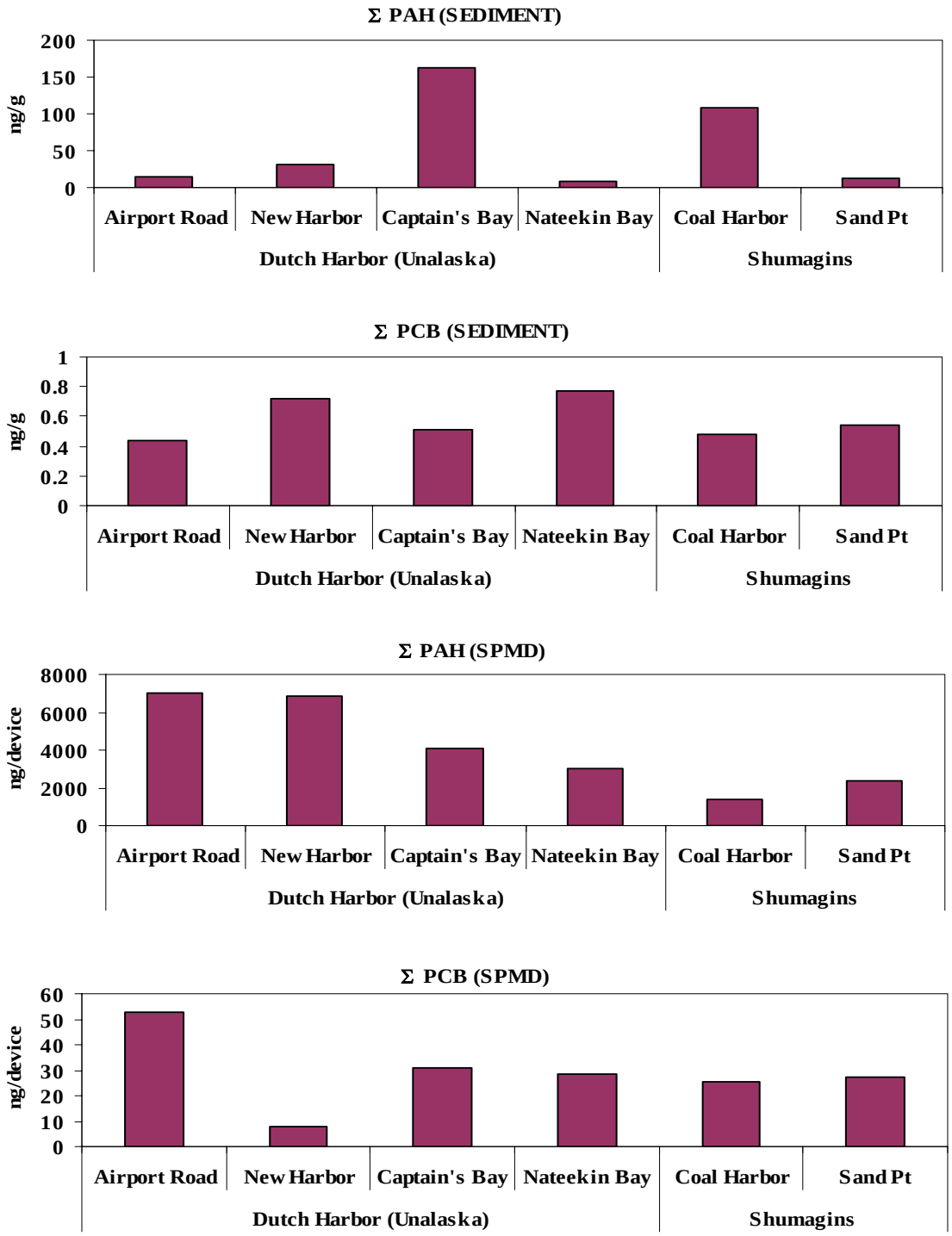


Figure 11. Concentrations of □ PAH and □ PCB in sediment samples and SPMD devices at sites in Unalaska and the Shumagin Islands, Alaska. Note y-axis scale differs among groups.

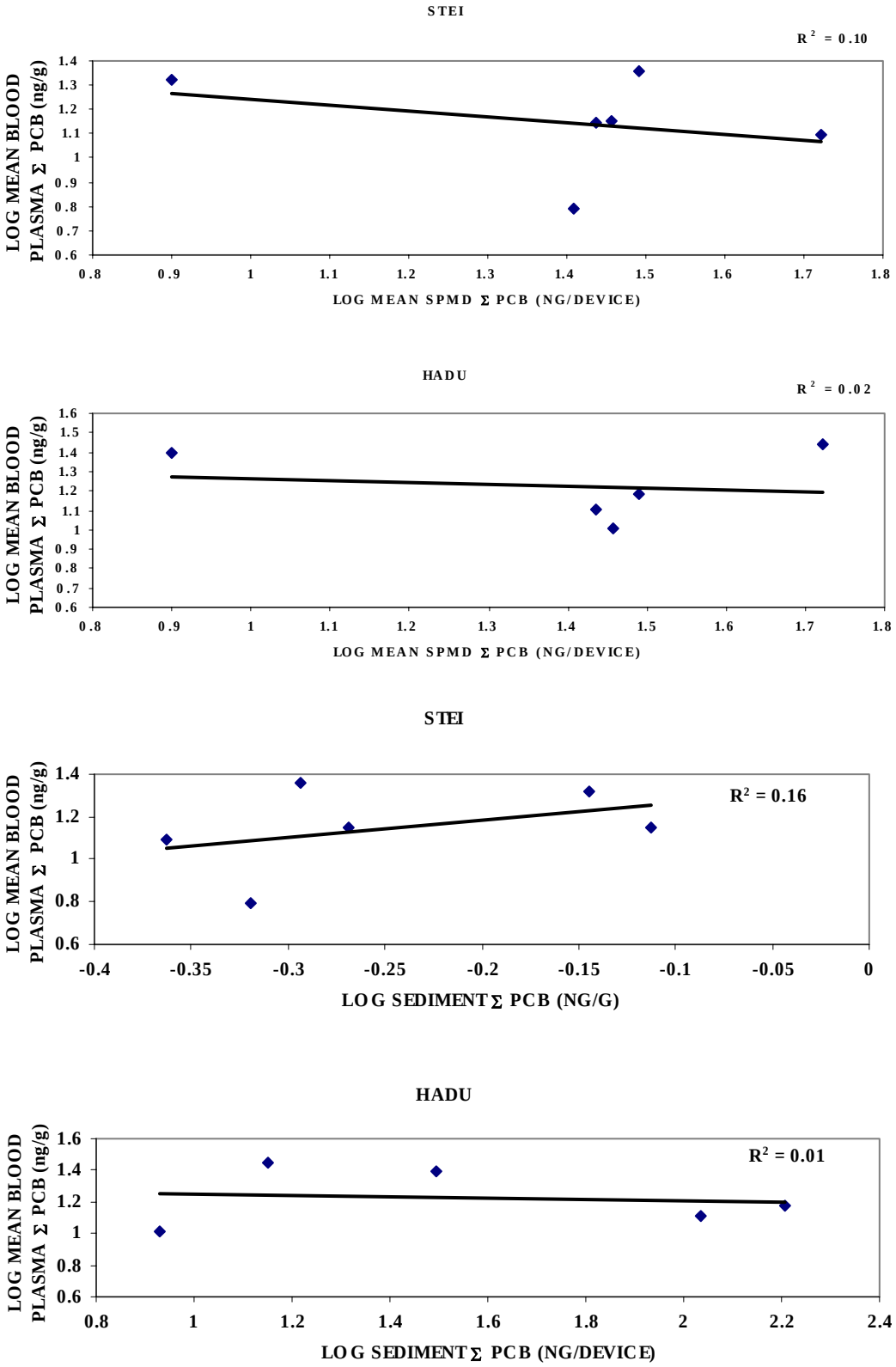
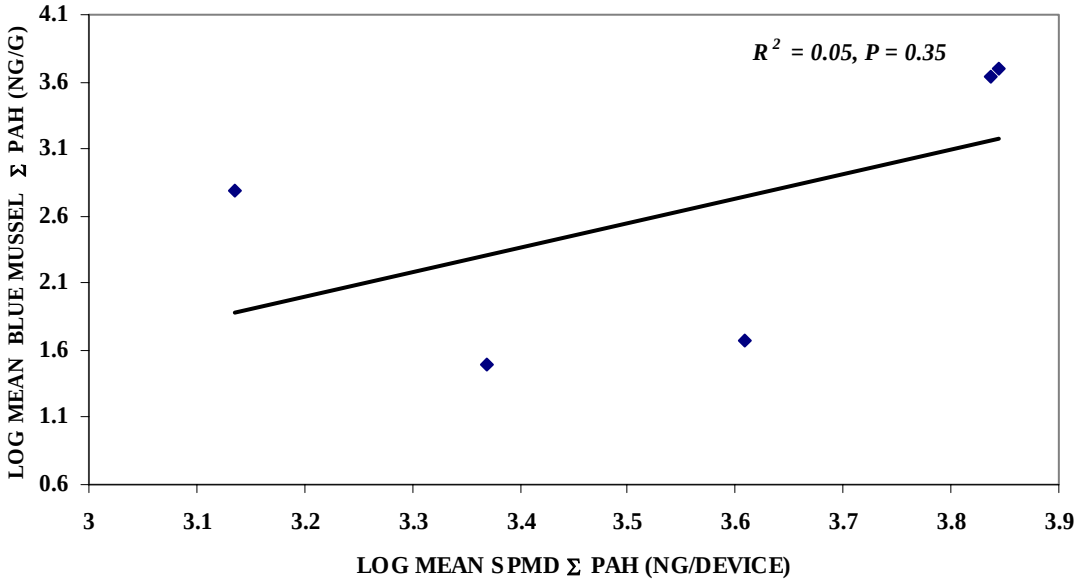


Figure 12. Correlations between concentrations of PCBs in blood plasma from STEI and HADU and PCBs in SPMD and sediment samples from sites at Unalaska and the Shumagin Islands, Alaska.



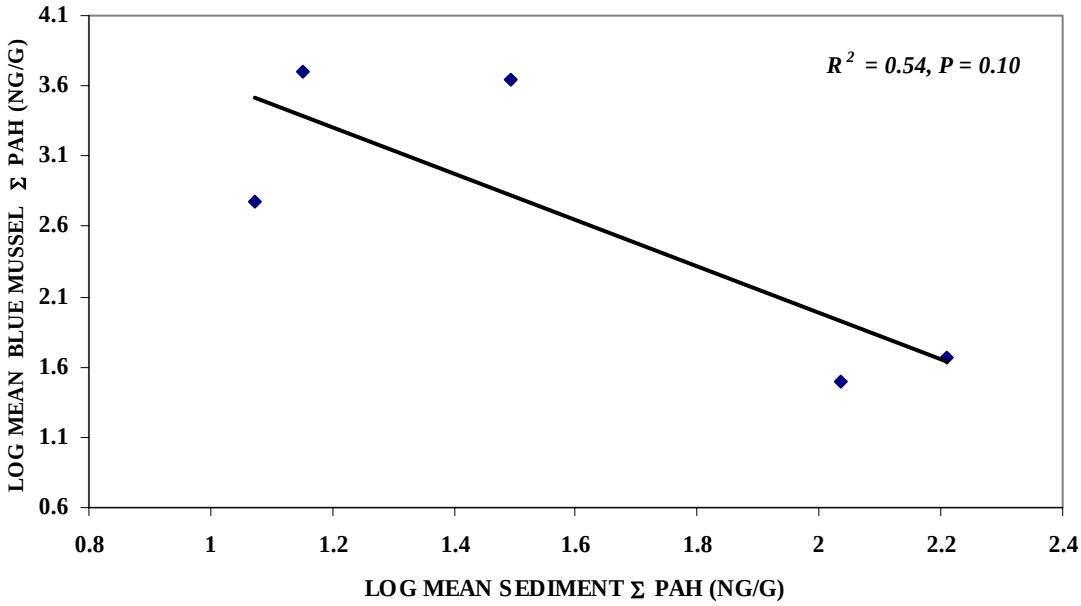


Figure 13. Correlations between concentrations of PAHs in blue mussels, and levels of PAHs in sediments and SPMD devices from sites at Unalaska and the Shumagin Islands, Alaska, 2002-2003.

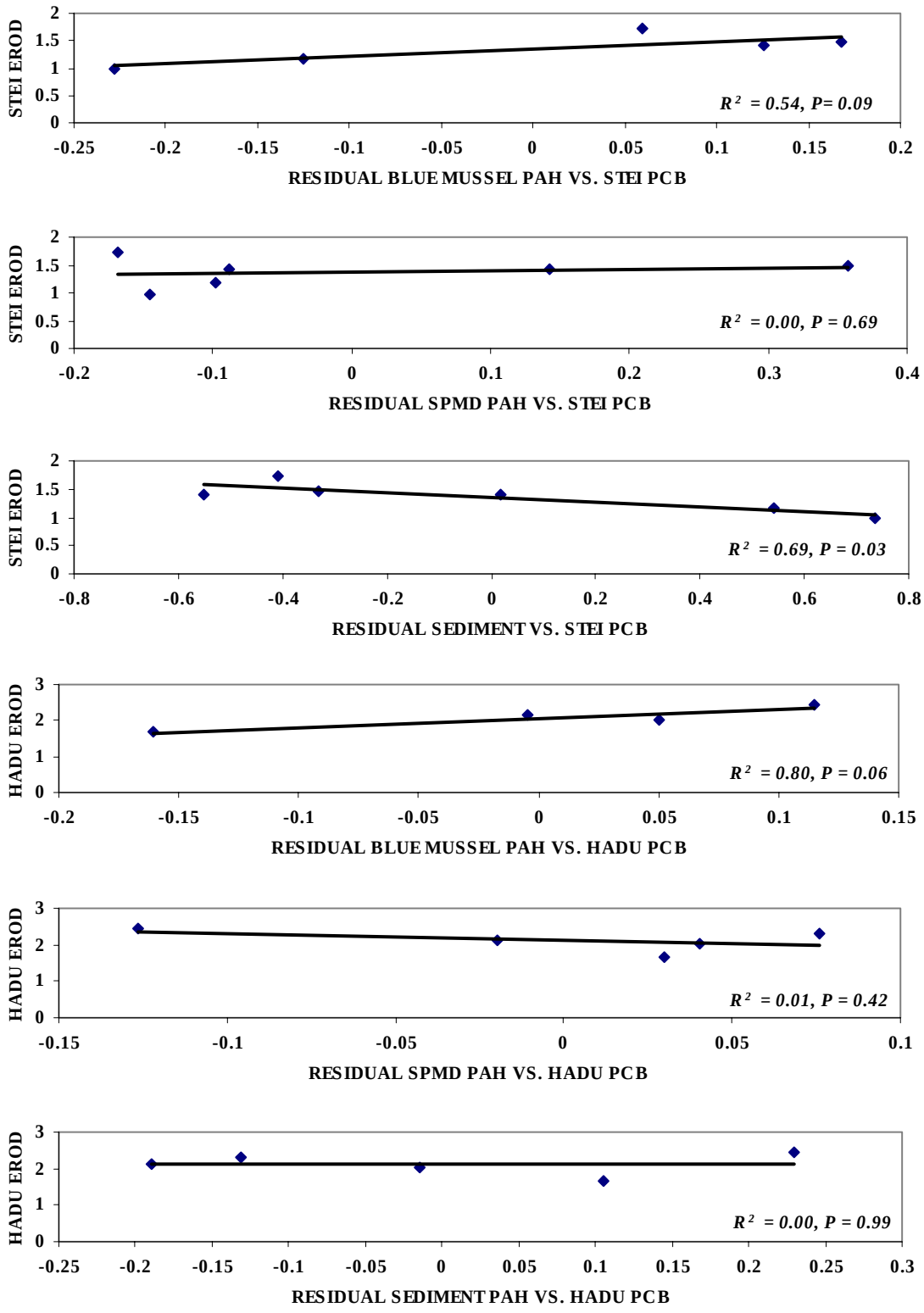


Figure 14. Relations between EROD activity in Stellers eiders and harlequin ducks and environmental PAHs while controlling for PCBs effects. Environmental PAHs were assumed to predict PAHs in waterfowl. Concentrations and EROD activity were log transformed.

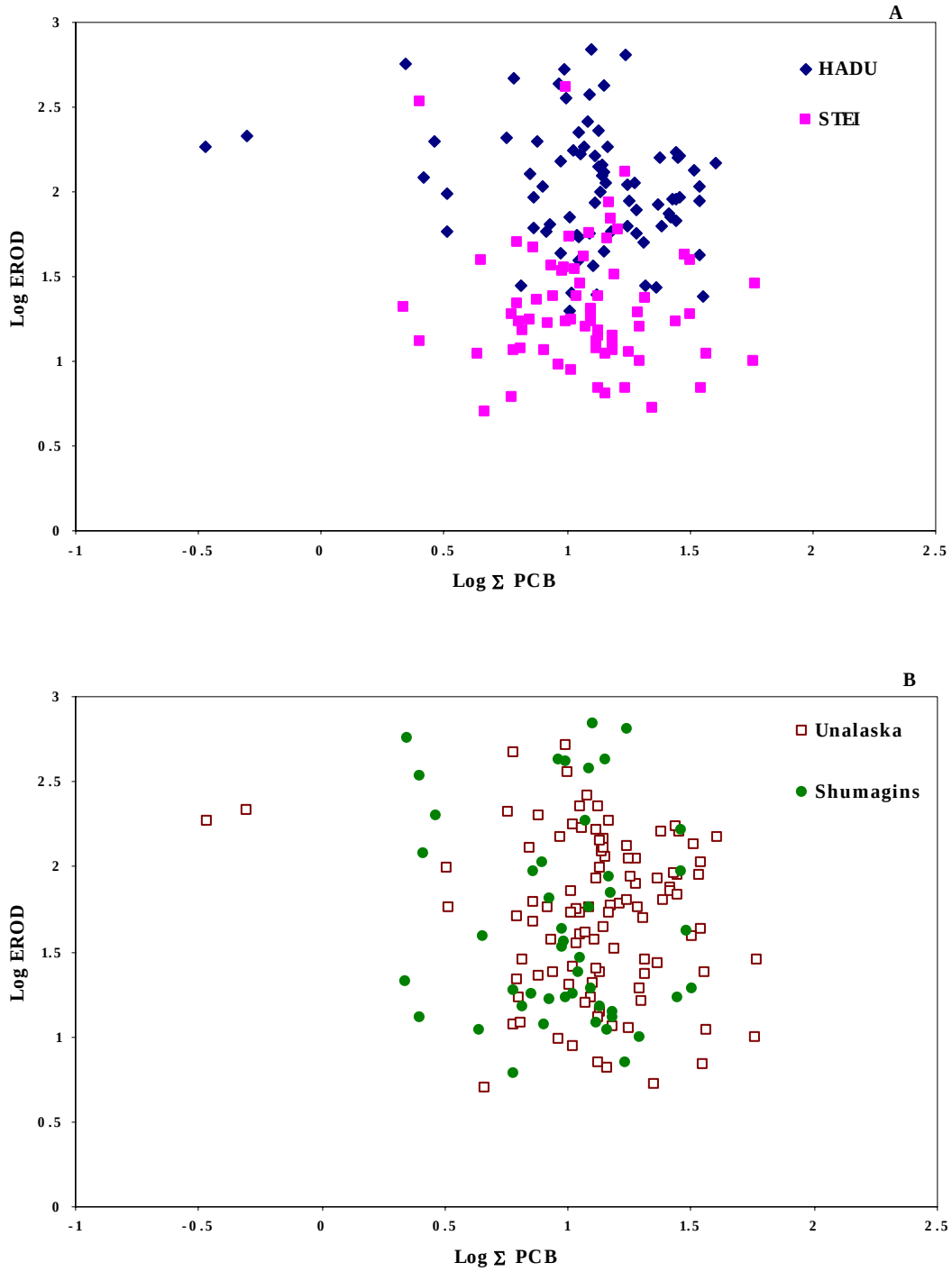


Figure15. Relation between log transformed EROD activity and S PCBs in seaducks while accounting for variation among species (A) and islands (B). Effect of PCBs on EROD was not significant (P = 0.14, partial R2 = 0.01).

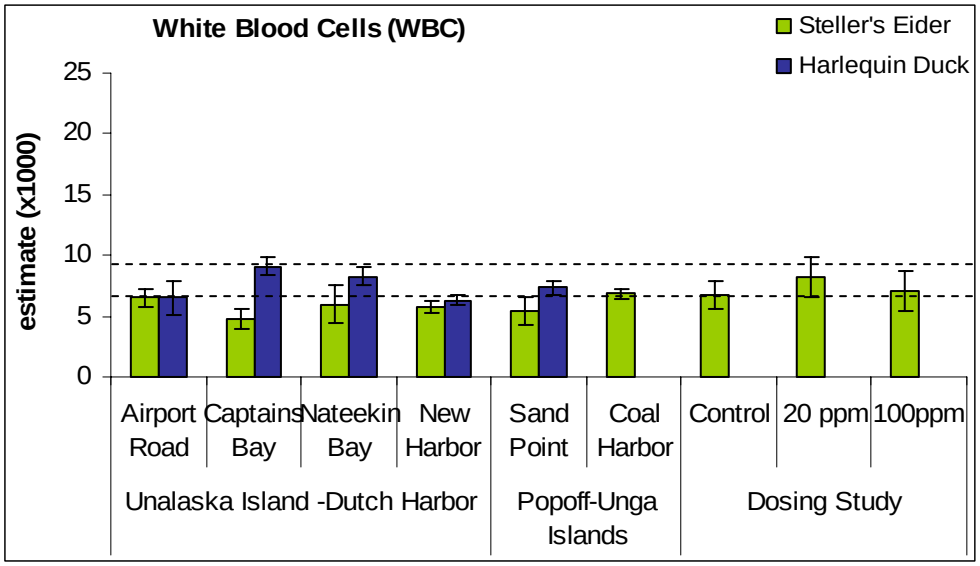


Figure 16. White Blood Cell estimates for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for WBC estimate in White-winged wood duck (4.7 – 9.4; Beynon 1996).

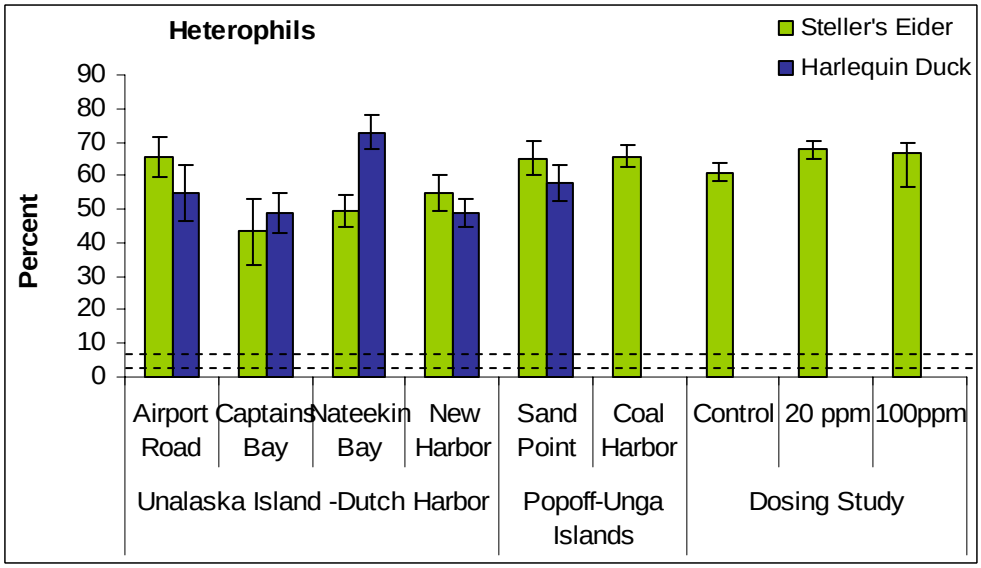


Figure 17. Heterophil counts for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for heterophils in American black duck (2.7 – 5.6; Johnson-Delaney and Harrison 1996).

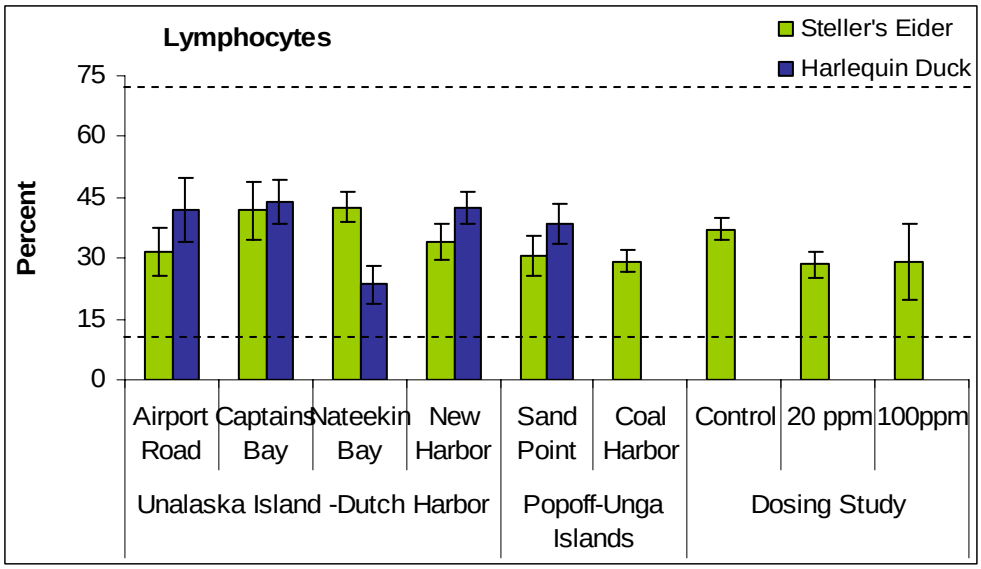


Figure 18. Lymphocyte counts for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for lymphocytes in ducks (13 – 73.5; Johnson-Delaney and Harrison 1996).

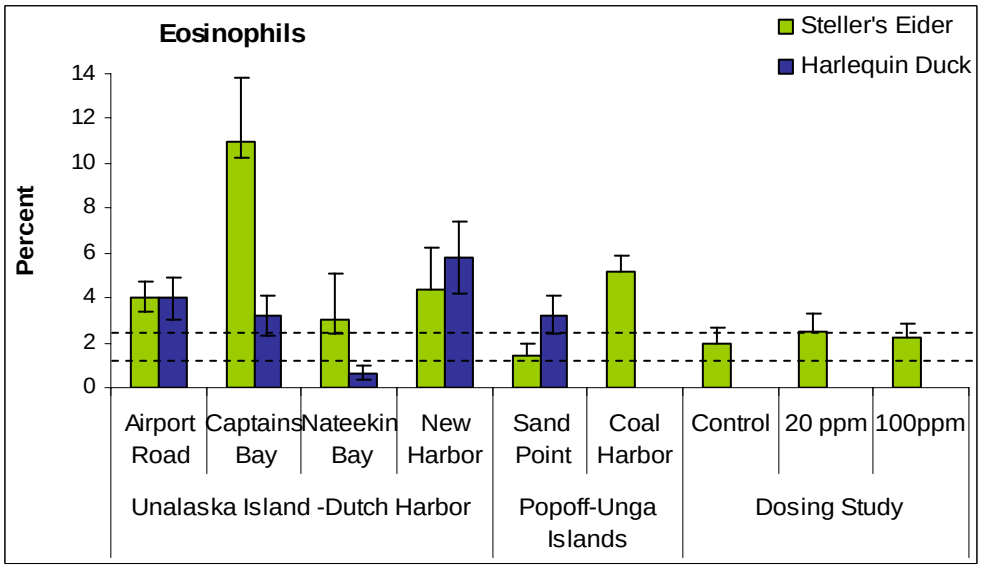


Figure 19. Eosinophil counts for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for eosinophils in ducks (1.6 – 2.65; Johnson-Delaney and Harrison 1996).

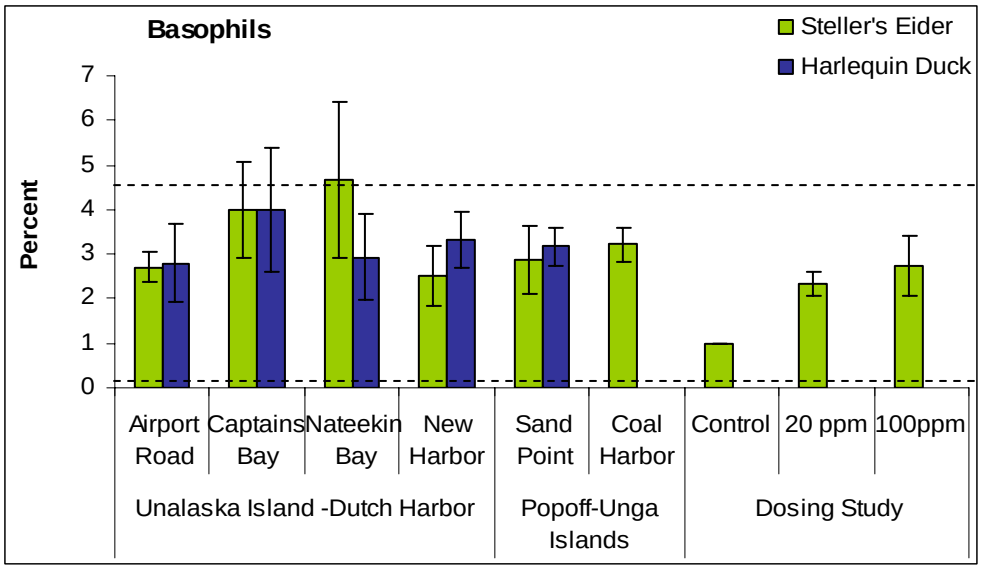


Figure 20. Basophil counts for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for basophils in ducks (0.00 – 4.5; Johnson-Delaney and Harrison 1996).

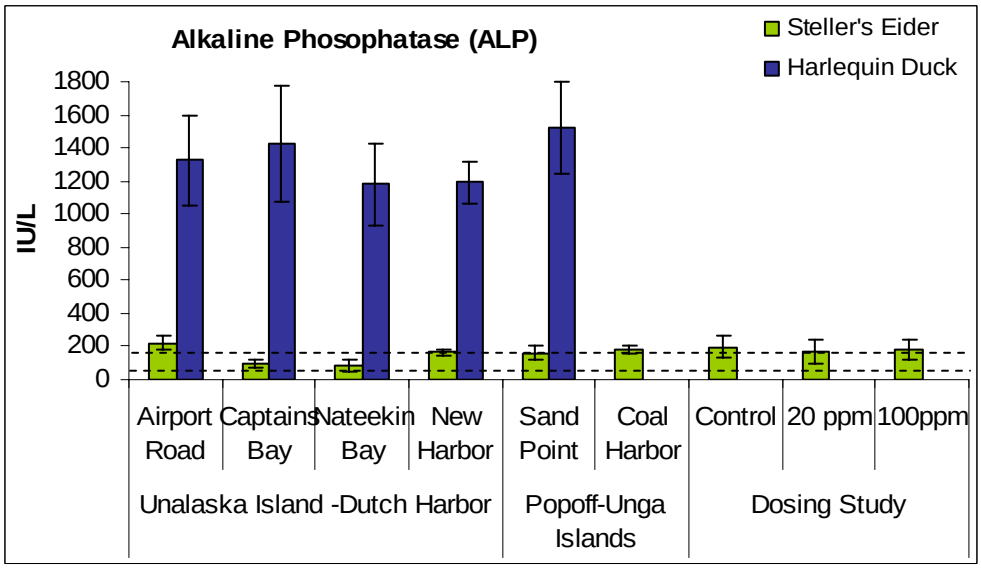


Figure 21. Alkaline Phosophatase levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for Alkaline Phosophatase in American black ducks (93.1 – 170.5; Franson 1982).

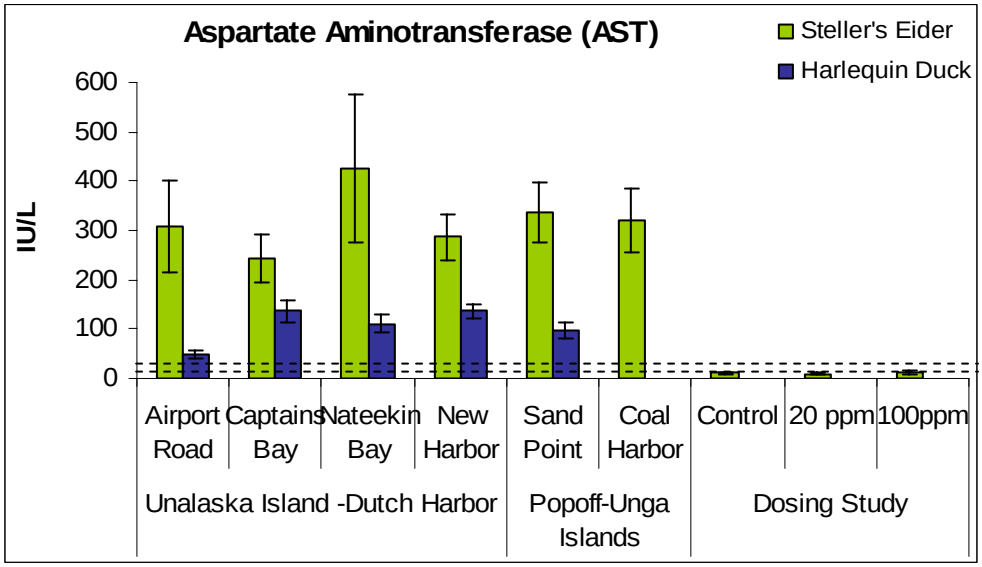


Figure 22. Aspartate Aminotransferase levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for aspartate aminotransferase in American black ducks (10.4 – 26.8; Franson 1982).

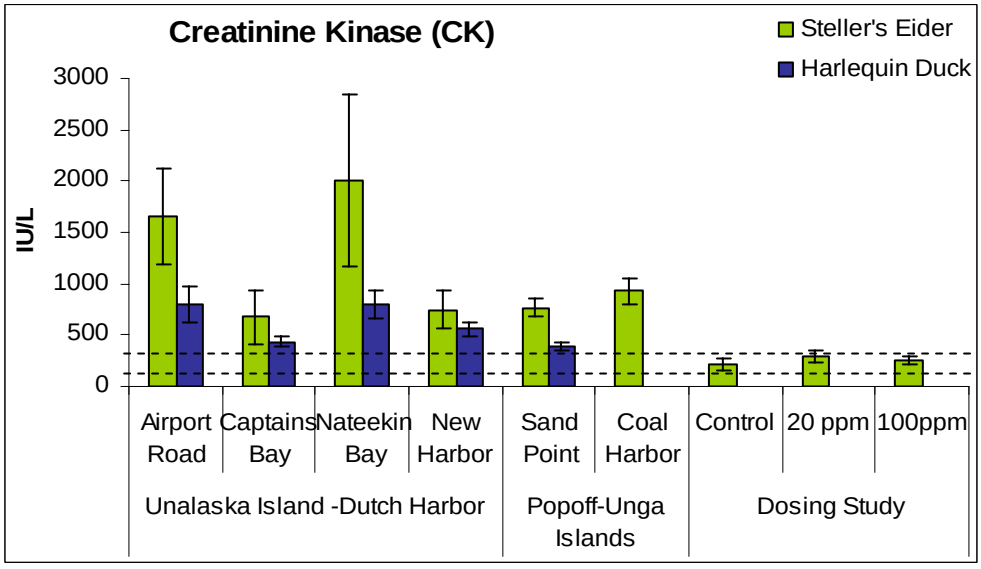


Figure 23. Creatinine Kinase levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for Creatine Kinase in adult Male Mallard ducks (210 – 350; Fairbrother 1990).

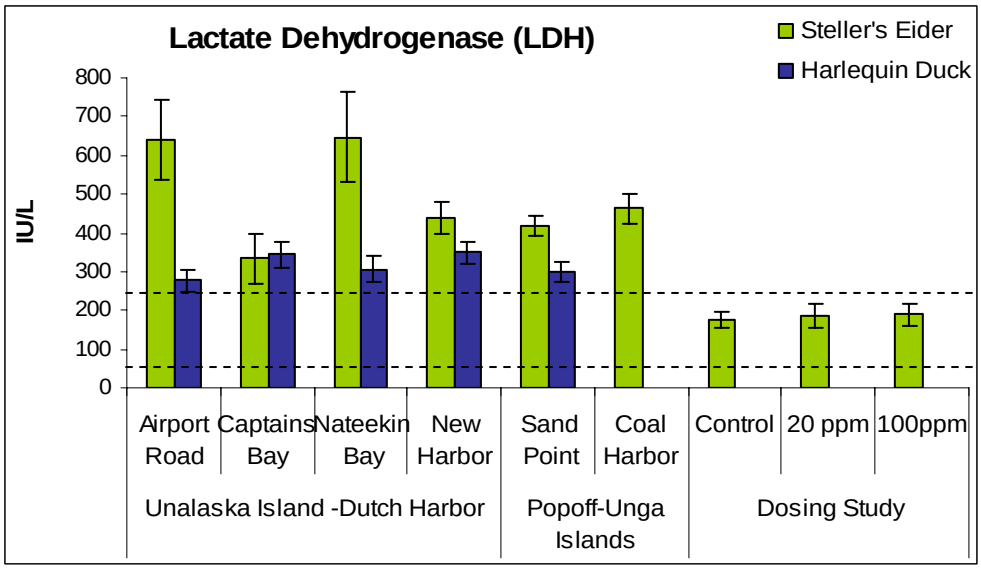


Figure 24. Lactate Dehydrogenase levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for Lactate dehydrogenase in adult Male Mallard ducks (67 – 227; Fairbrother 1990).

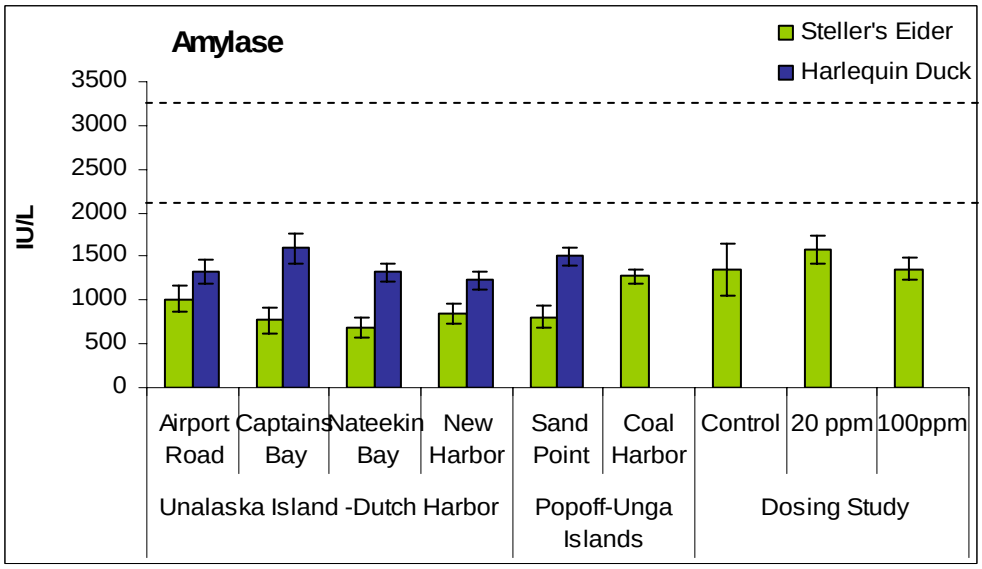


Figure 25. Amylase levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for amylase in adult Male Mallard ducks (2001 – 3261; Fairbrother 1990).

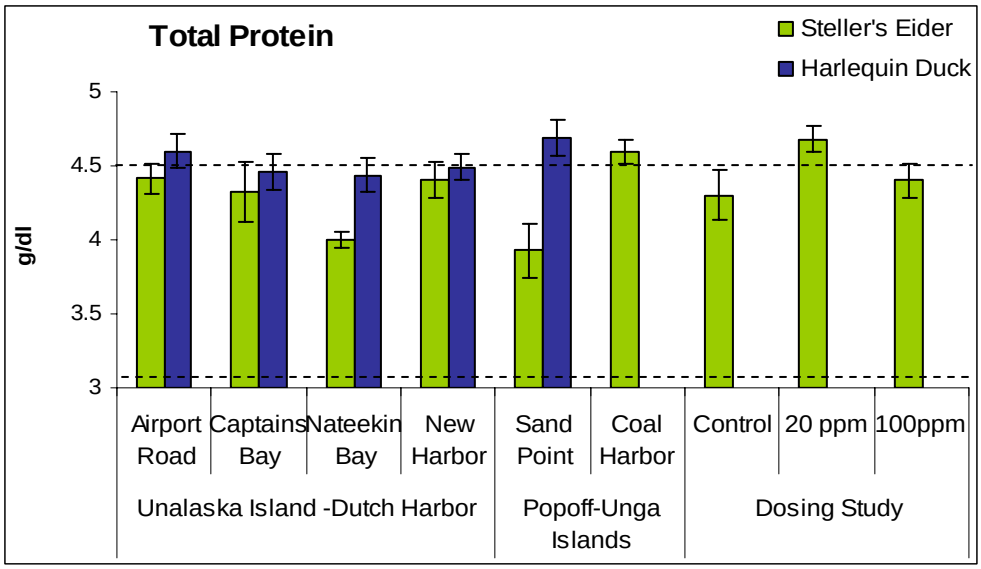


Figure 26. Total protein levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for total protein in adult Male Mallard ducks (3.1 – 4.5; Fairbrother 1990).

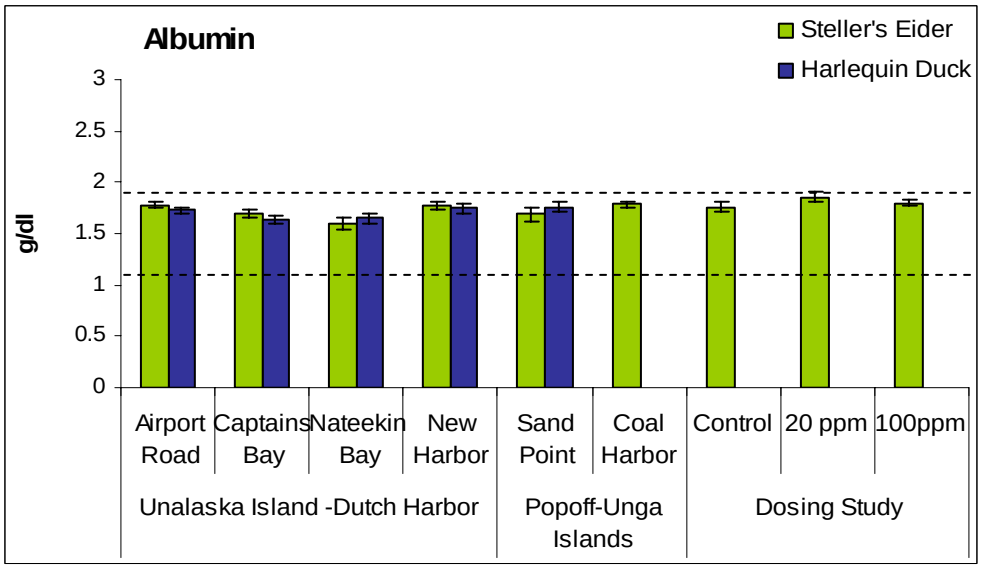


Figure 27. Albumin levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for albumin in adult Male Mallard ducks (1.1 – 1.9; Fairbrother 1990).

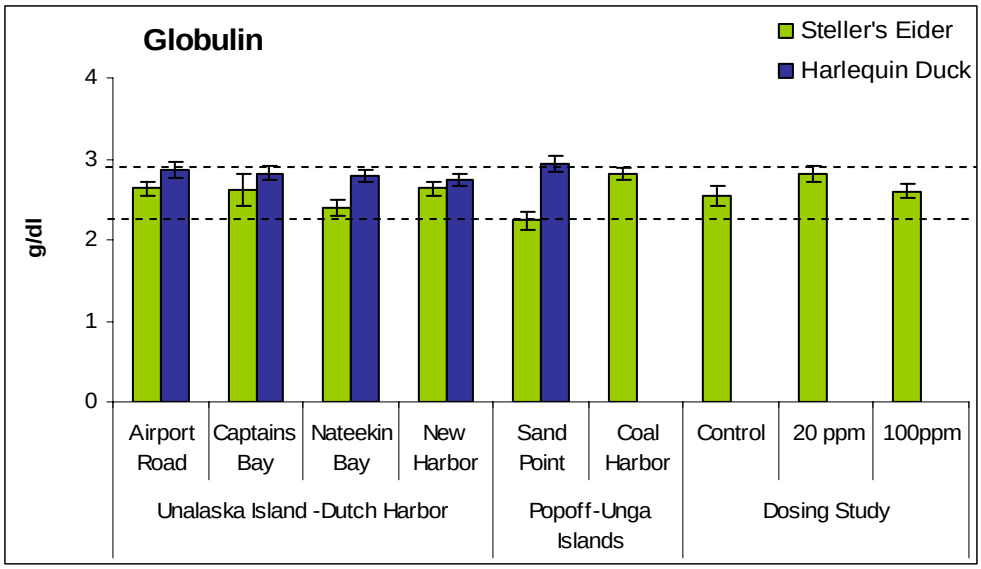


Figure 28. Globulin levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for globulin in Peking ducks (2.3 – 2.9; Spano 1987).

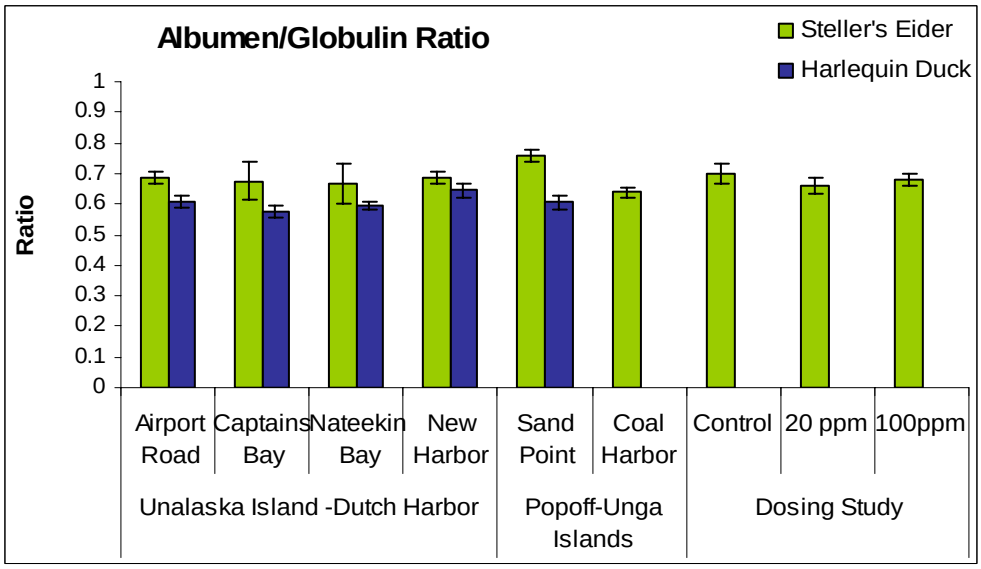


Figure 29. A:G ratio levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004.

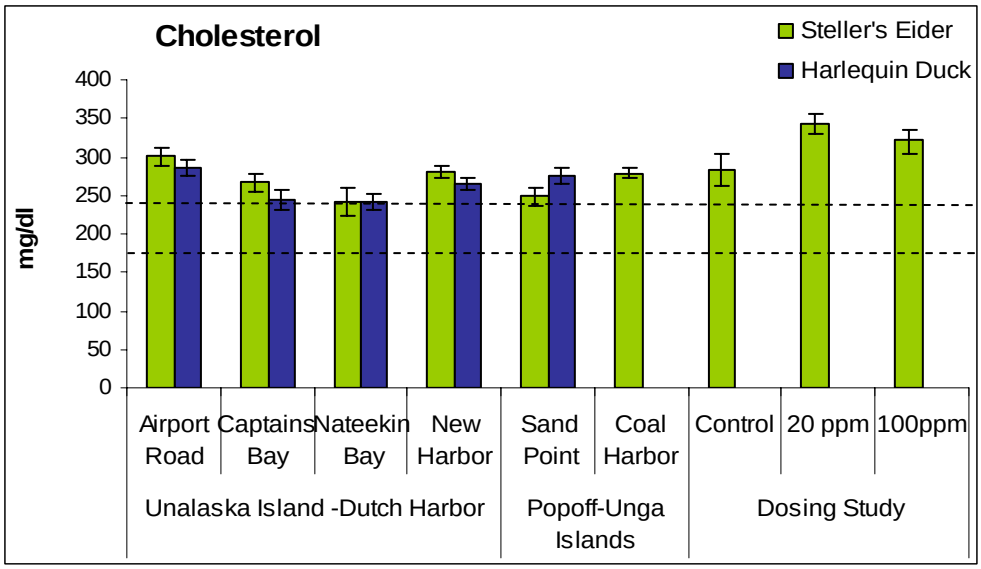


Figure 30. Cholesterol levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for cholesterol in Peking ducks (176.4 – 247.6; Spano 1987).

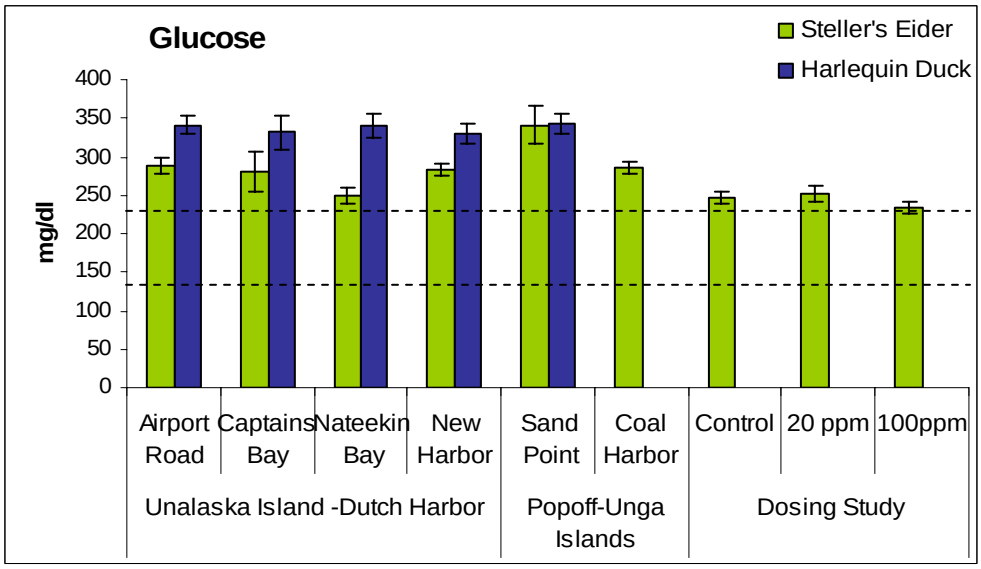


Figure 31. Glucose levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for glucose in adult Male Mallard ducks (138 – 232; Fairbrother 1990).

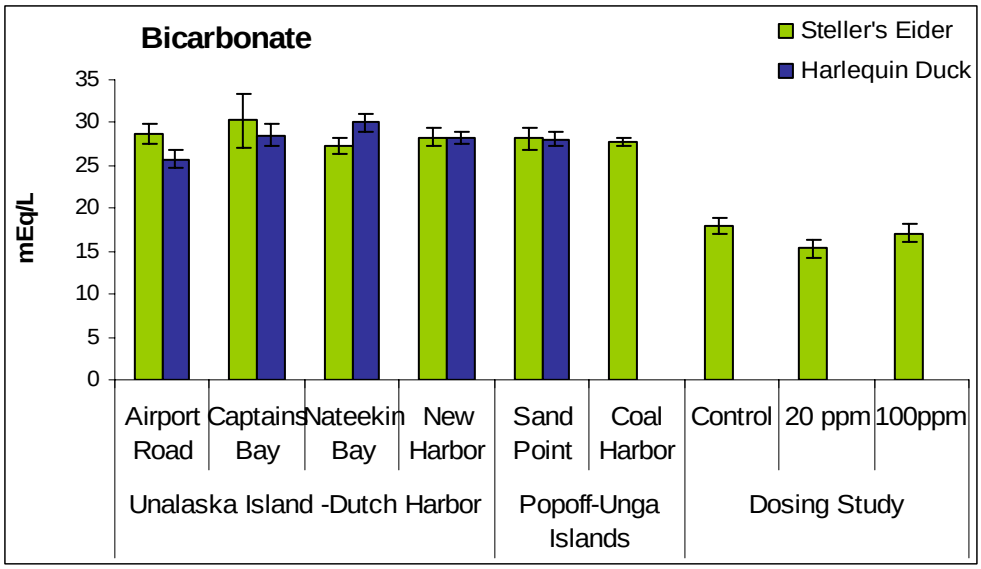


Figure 32. Bicarbonate levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004.

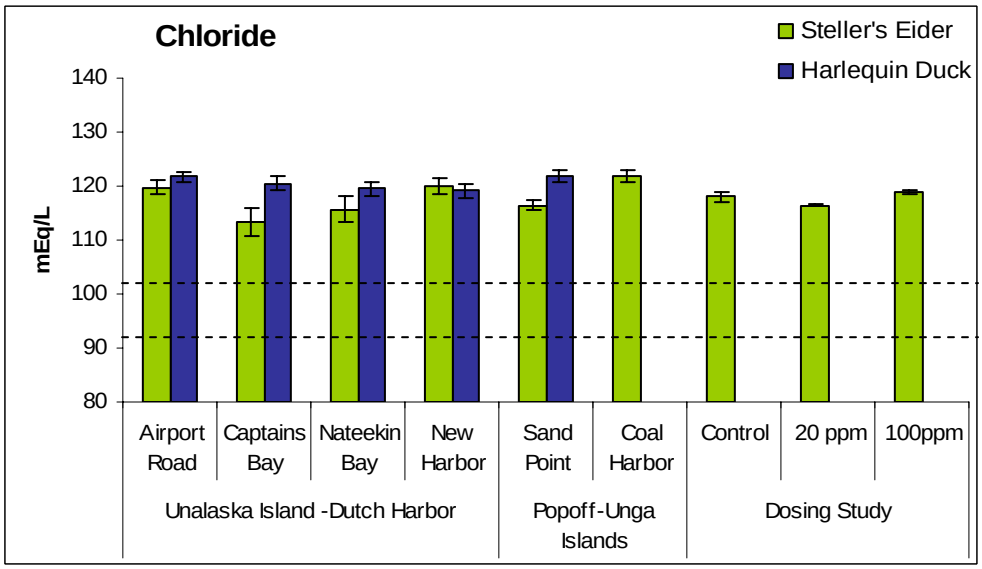


Figure 33. Chloride levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for chloride in Peking ducks (92 – 102; Spano 1987).

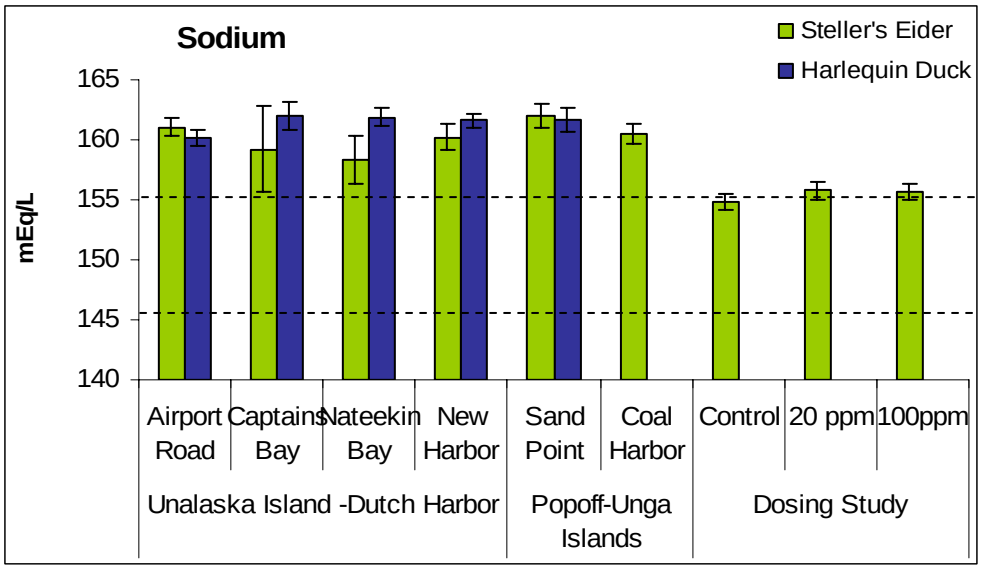


Figure 34. Sodium levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for sodium in Peking ducks (146.8 – 155.2; Spano 1987).

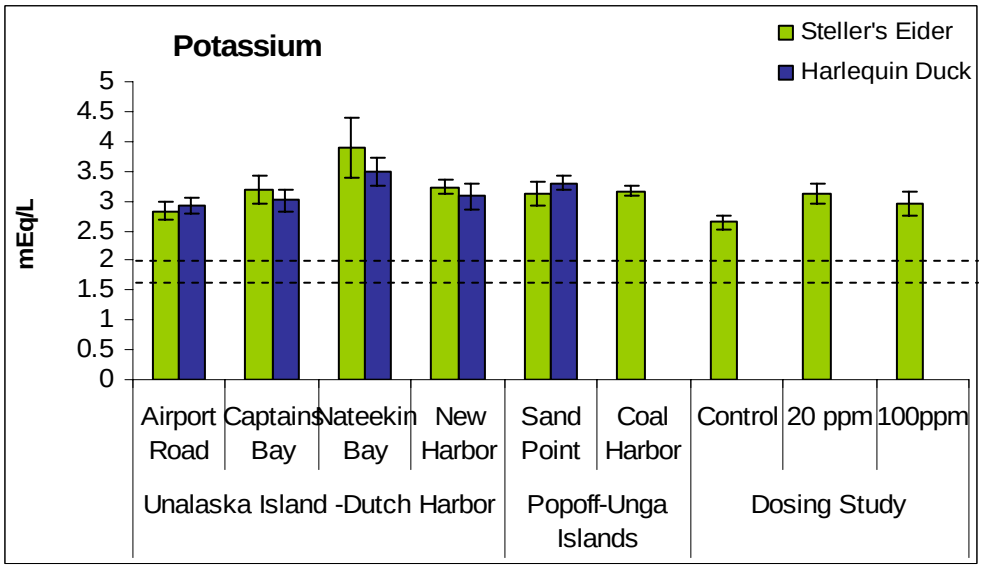


Figure 35. Potassium levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for potassium in Peking ducks (1.6 – 2.0; Spano 1987).

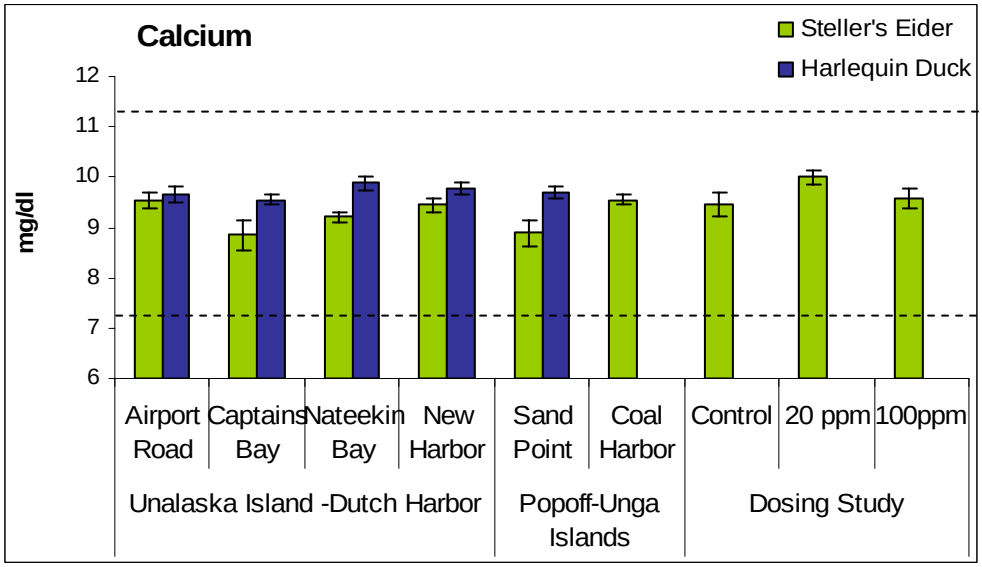


Figure 36. Calcium levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for calcium in adult Male Mallard ducks (7.5 – 11.3; Fairbrother 1990).

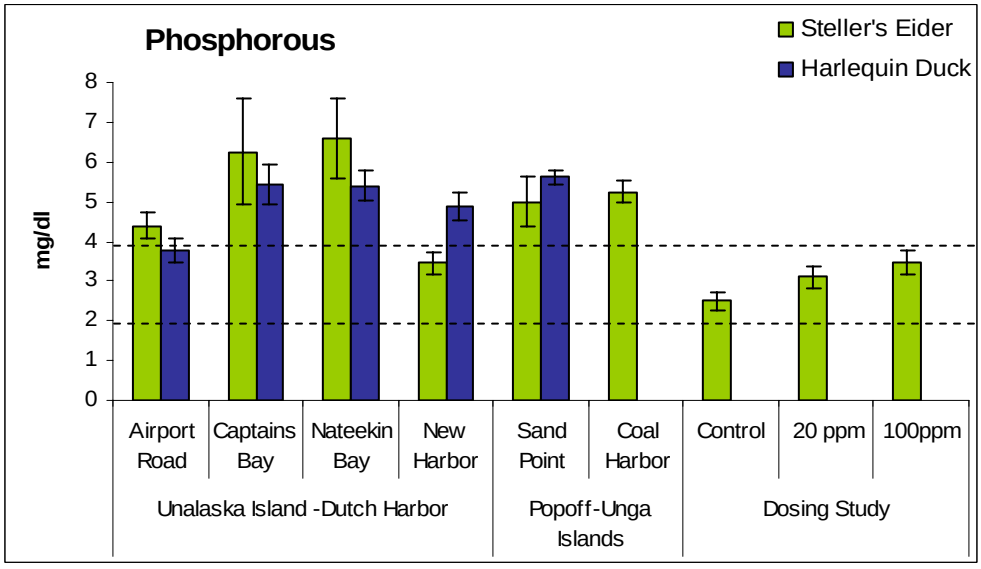


Figure 37. Phosphorous levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for phosphorous in adult Male Mallard ducks (1.9 – 3.9; Fairbrother 1990).

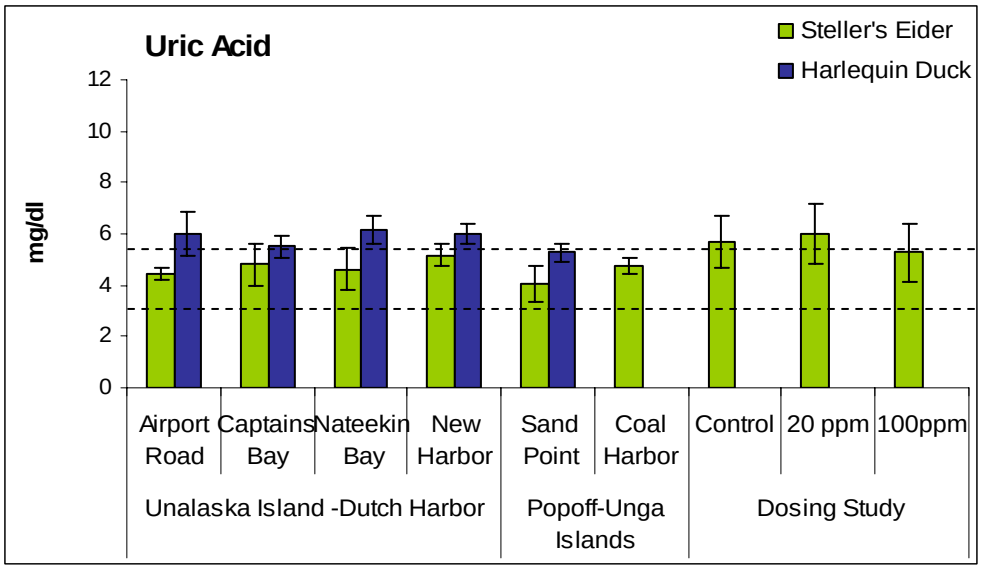


Figure 38. Uric acid levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for uric acid in adult Male Mallard ducks (2.7 – 5.3; Fairbrother 1990).

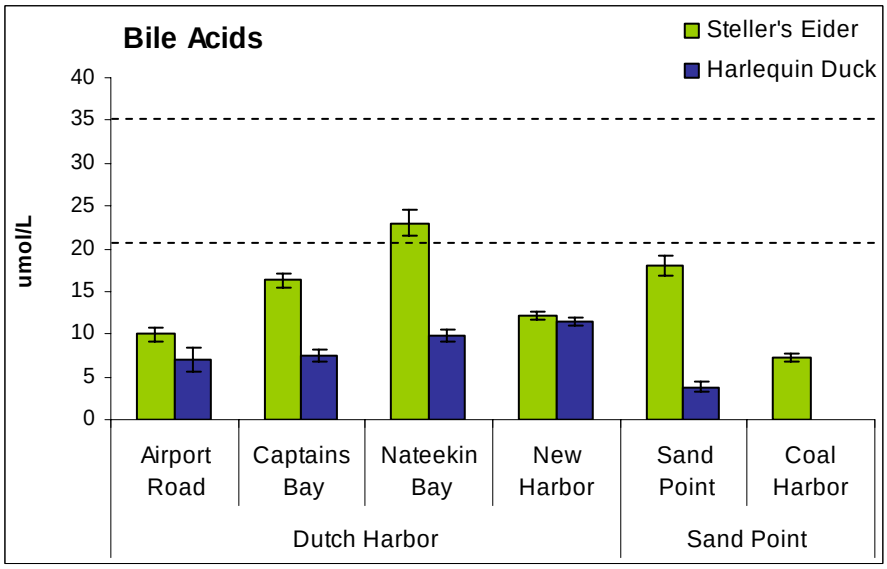


Figure 39. Bile acid levels for STEI and HADUs sampled at Unalaska, Dutch Harbor and Popoff Unga Islands, winter 2002-2003, as well as captive STEI winter 2004, dashed line indicates baseline range for bile acids in adult male Mallard ducks, fasting levels (20.2 – 35.4; Fowler and Miller 2003).

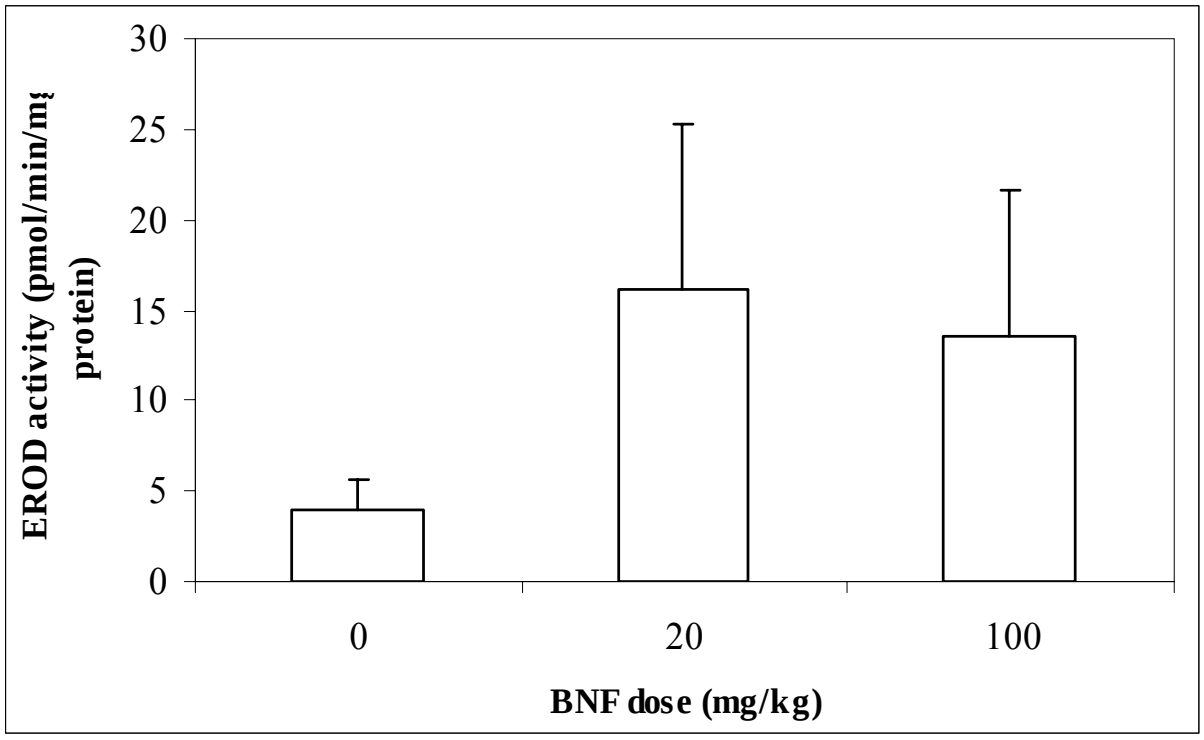


Figure 40. EROD activity in captive dosed STEI collected from Unalaska, Dutch Harbor winter 2004.

Table 1. Number of samples analyzed for EROD, PCBs, aromatic and aliphatic hydrocarbons, and chlorinated pesticides for the Steller's eider study.

			number of samples analyzed				
Pooled Species	Island	SITE	EROD [a]	PCBs	Aromatic Hydrocarbons (PAH)	Aliphatic Hydrocarbons	Chlorinated Pesticides (OC)
Steller's Eider	Unalaska	Airport Road	15	15			
	Unalaska	Captain's Bay	4	4			
	Unalaska	Nateekin Bay	3	3			
	Unalaska	New Harbor	16	16			
	Shumagins	Coal Harbor	7	7			
	Shumagins	Sand Pt	26	32			
Harlequin Duck	Unalaska	Airport Road	12	14			
	Unalaska	Captain's Bay	13	13			
	Unalaska	Nateekin Bay	16	16			
	Unalaska	New Harbor	25	26			
	Shumagins	Sand Pt	15	19			
Black Scoter	Unalaska	Nateekin Bay	1	1			
	Unalaska	New Harbor	2	2			
Blue mussel	Unalaska	Airport Road			6	6	6
	Unalaska	Captain's Bay			3	3	3
	Unalaska	New Harbor			3	3	3
	Shumagins	Coal Harbor			3	3	3
	Shumagins	Sand Pt			3	3	3
Crustaceans	Unalaska	Airport Road			4		4
	Unalaska	Captain's Bay			2		2
	Unalaska	New Harbor			2		2
Tegula	Shumagins	Coal Harbor			3		3
	Shumagins	Sand Pt			3		3
Flocculi	Unalaska	New Harbor			3		3
	Shumagins	Sand Pt			3		3
Sediment	Unalaska	Airport Road		1	1		
	Unalaska	Captain's Bay		1	1		
	Unalaska	Nateekin Bay		1	1		
	Unalaska	New Harbor		1	1		
	Shumagins	Coal Harbor		1	1		
	Shumagins	Sand Pt		2	2		
SPMD	Unalaska	Airport Road		1	1		
	Unalaska	Captain's Bay		1	1		
	Unalaska	Nateekin bay		1	1		
	Unalaska	New Harbor		1	1		
	Shumagins	Coal Harbor		1	1		
	Shumagins	Sand Pt		2	2		

[a] does not include samples defrosted during shipping

APPENDIX A:

Movement and Home Range of Wintering Steller's Eiders near Dutch Harbor, Alaska, February and March 2004

MOVEMENT AND HOME RANGE OF WINTERING STELLER'S EIDERS NEAR DUTCH HARBOR, ALASKA, FEBRUARY AND MARCH 2004

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INTRODUCTION

Declines of Steller's eiders (*Polysticta stelleri*) on their breeding areas in Alaska have resulted in the North American breeding population being listed as *Threatened* under the provisions of the Endangered Species Act (Fox *et al.* 1997, Flint *et al.* 2000). The Pacific population of Steller's Eiders breed across Arctic Alaska and Siberia and winter along the Alaska Peninsula and Aleutian Islands. Factors related to the population decline are unknown and may be occurring on breeding areas, wintering areas, or both. Winter has been described as a critical period in the annual cycle for sea ducks, as these birds typically winter at relatively high latitudes where days are short and metabolic costs are high (Camphuysen *et al.* 2002).

Activities such as boat harbor developments and increased seafood processing may impact eiders that winter along the Alaska Peninsula and eastern Aleutian Islands. Some Steller's Eiders winter in areas of industrial development near seafood processing plants and associated boat harbors (Hoffman 2000, 2001). Eiders may use these areas because of the presence of ice-free waters that are typically well protected from high energy wave action. Alternatively, sea ducks may be attracted to the increased primary productivity resulting from eutrophication associated with seafood processing wastewater and municipal sewage discharges (Campbell 1984). However, industrial activities may displace birds from foraging sites, destroy natural foraging habitat, or expose birds to contaminants. Further, the eutrophication itself may increase up-take of contaminants such as PCB's and petroleum-based products into the food chain (Persson *et al*. 1995, Dachs *et al*. 2000, Gunnarsson *et al*. 2000, Skei *et al*. 2000.). Thus, these industrial areas may function as 'traps' that attract wintering seaducks, which are then exposed to harmful levels of contaminants. Information on Steller's Eiders and their wintering site fidelity, home range, and migration patterns is limited. Understanding patterns of site

fidelity, home range, and habitat use by wintering populations is essential to predicting the effect of disturbance, habitat loss, or exposure to contaminants.

Our objectives during the winter of 2004 were to assess habitat use, home range size, and behavior of Steller's Eiders and compare these parameters with sympatric Harlequin Ducks in the vicinity of Dutch Harbor. We interpret these results in relation to habitat conditions, predator disturbance, point sources of pollution, and proposed industrial developments.

METHODS

Between 26 January and 3 February 2004, we captured ducks at five sites in the vicinity of Dutch Harbor, Alaska (53° 53' N, 166° 33' W). Sites included: 1) Hog Island, 2) Airport Beach just south of the runway, 3) the bay along Airport Beach Road adjacent to the outfall sites for the municipal sewage system and seafood processing waste, 4) Little South America (the area proposed for a harbor development), and 5) Captain's Bay near Port Levashef (Figure 1). We used floating mist nets with decoys to capture both species (Kaiser *et al.* 1995).

All captured birds were banded, weighed, sexed, and aged. We attached 10 g radio transmitters using the sub-cutaneous prong and suture transmitter attachment described by Peitz *et al.* (1995). Birds were released at the capture location. We tracked radio-marked ducks from 26 January through 26 March with peak-null antenna systems mounted in trucks. We used 2 different approaches to monitor movements and habitat use. First, given the access on the road system, a single observer could establish the location of an individual within a series of four zones (Figure 1). These zones are arbitrary in biological terms and are based on geography and our ability to accurately define radio transmitter locations. Using this approach we scanned for all transmitters multiple times per day. For every transmitter detected, we recorded the time, location, strength of signal, and activity (diving or roosting). Second, between 22 February and 26 March we collected simultaneous bearings from trucks in two locations for all radios that provided clear signals to both trucks three times per day. We then used the bearing data to calculate more precise locations using simple triangulation (White and Garrott 1990).

ANALYSES

The procedure for estimating locations based on triangulation allows estimation of the error associated with point estimates (White and Garrott 1990). We eliminated triangulated locations with error polygons > 95th percentile of all locations (75 ha) as well as point locations

falling on land. The median error polygon size of our remaining dataset was 0.61 ha. We used program Animal Movement (Hooge and Eichenlaub 2000) to calculate Minimum Convex Polygons (MCP) of triangulated points as an index of home range size. As these sea ducks do not use onshore habitats during the winter, we subtracted all land area that fell within individual MCPs from the final area calculation.

We estimated within day movement probabilities by calculating the proportion of bird days with multiple locations where birds were found in more than one zone. We pooled birds within days and estimated the standard error of this proportion among days. We then used zone level locations in multi-state mark-recapture models to estimate the detection and movement probabilities among zones, across days for each species (Flint *et al.* 2004).

We estimated time spent foraging using signal patterns from radio transmitters. When birds are diving radio signals are interrupted for 10-30 seconds because salt-water attenuates radio signals. Thus, observers recorded if birds were diving (i.e., foraging) for each observation. We lumped daylight hours into 3-4 hour blocks and calculated the proportion of birds detected as diving during each time block. At the time of this study, dawn occurred at about 0900 and dusk about 2000.

RESULTS

Between 26 January and 3 February we captured and radio-marked 28 Steller's eiders (8 male, 20 female) and 32 Harlequin ducks (22 male, 10 female). We detected all 60 radio-marked ducks after release. During February we recovered twelve transmitters (six per species) from dead birds or shed from live birds. Of the remaining transmitters all Steller's eiders were detected through February and all but four were detected through 20 March. Only two Harlequin ducks were not heard through 20 March. The mean number of detections per Steller's eider was 214.4 (SE = 25.4) and per Harlequin duck was 192.3 (SE = 21.7) (Table 1).

To estimate home range size, we calculated minimum convex polygons for each radio-marked duck for which we had triangulated locations on at least five different days (eiders n = 18, Harlequin ducks n = 20). None of the 12 mortalities/shed radios mentioned above were included in the calculations. Mean MCP was slightly larger for Steller's eiders (37.9 ha ± 7.8 SE) than Harlequin ducks (30.3 ha ± 5.2 SE). Within species, individual home range sizes varied considerably, but most encompassed at least two zones (Fig. 2*a* & *b*).

The probability of moving from one zone to another on subsequent days was highly variable, ranging from 0-92% for eiders and 0-65% for Harlequin ducks (Table 2a & b) with shorter movements generally more likely. These same models estimated detection probabilities between 0.39 and 0.97 (range among zones) for Steller's Eiders and 0.31 and 0.46 for Harlequin Ducks. We did not have sufficient data to estimate specific movement probabilities between each zone within days, but we were able to generate an overall probability of being located in multiple zones during a single day (Steller's eider = 40.5 ± 4.1 %, Harlequin duck = 39.6 ± 3.8%).

Harlequin Ducks were determined to be foraging more often than Steller's Eiders (Figure 3). Using these estimates of percent time foraging within time blocks, we estimate that Steller's Eiders spent 2.27 hours foraging per day compared to 4.18 hours for Harlequin Ducks. Both species fed primarily during daylight hours (Figure 3). Steller's Eiders showed a slight diurnal pattern with higher probabilities of foraging at dawn and dusk. Further, it appears that Steller's Eiders foraged more at night than Harlequin Ducks. For Steller's Eiders, night-time foraging was more common after dusk than before dawn.

DISCUSSION

Home range and Movement Patterns

Our data suggest that both Steller's Eider and Harlequin Ducks are highly mobile while wintering around Dutch Harbor. Our home range estimates, though variable, are fairly large and commonly encompassed several of our arbitrary zones. Further, we believe we underestimated home range size. The mark-recapture models we used estimate the detection probability which is defined as the probability that a marked individual present on the study area is actually detected on a given day. However, in our application, presence of the radio transmitters should have been detected within these zone, if they were present, at rates very close to 1.0. Detection probability will be biased low if marked individuals show temporary immigration where they depart the study area for a period of time to a location where they are not detected, then return to the study area (Pollock *et al.* 1990). Thus, given our relatively low detection probabilities, we believe that marked individuals regularly moved beyond the range of our tracking system which would result in underestimation of home range size. We know from visual observations that concentrations of both species can occasionally be found on the northwest side of Hog Island, the north side of Ballyhoo, and in Nateekin Bay (areas adjacent to our study area that are beyond our

detection range). In addition to large home range size, movement probabilities (both daily and within days) were high. Commonly, depending on the area, one-third to one-fifth of the birds in a zone are likely to move to another zone that day or the next.

This is the first study documenting home range size for wintering Steller's Eiders. Laubhan and Metzner (1999) reported that Steller's Eiders changed wintering areas in response to sea ice. However, ice was not present around Dutch Harbor during our study. Studies of Steller's Eiders wintering in Norway indicate that birds use similar habitats to those used in Dutch Harbor (shallow benches or kelp beds) (Fox and Mitchell 1997*a*, Bustnes and Systad 2001*b*), but report no data regarding movements or home ranges. Thus, we do not know if the home range size we report is unusual for wintering Steller's Eiders.

Conversely, Harlequin Ducks are thought to maintain relatively small home ranges during winter. Robertson *et al.* (1999) describe individual birds as using areas of only several hundred meters of coastline. In contrast, our data indicate that Harlequin Ducks maintain considerably larger home ranges in the vicinity of Dutch Harbor regularly moving across several kilometers of open water. Thus, Harlequin Ducks at Dutch Harbor appear unusual in their home range size and movement patterns.

Foraging Behavior

Both Steller's Eiders and Harlequin Ducks in the Dutch Harbor area spent very little time foraging. Steller's Eiders wintering in Norway spent > 80% of their time foraging in late winter (Systad and Bustnes 2001) compared to <20% of the time at Dutch Harbor. Similarly, Harlequin Ducks wintering in Newfoundland spent 69% of daylight hours foraging (Goudie and Ankney 1986) compared to <40% at Dutch Harbor. Further, Systad and Bustnes (2001) reported that Steller's Eiders fed primarily at low tide in Norway. In contrast, foraging behavior at Dutch Harbor was not strongly related to tide stage for either species. However, maximum predicted tidal flux during the period of our study was only 5.6 feet, with almost all of the low tides late in the day or after dark. Our data suggest that eiders did take advantage of these low tides as there was more foraging after dark in the evenings than before light in the mornings. Harlequin Ducks fed relatively uniformly throughout the daylight period.

There are 2 competing hypotheses to explain the low rates of feeding at Dutch Harbor. First, Goudie and Ankney (1986) suggest that wintering sea ducks reduce energetically

expensive foraging behaviors such as diving when forage is limiting. Thus, wintering sea-ducks appear to modify their behavior in response to energetic cost-benefit trade-offs. Alternatively, high energy forage may be readily available such that birds are able to meet their daily energetic requirements very quickly. In support of this hypothesis the mean weights of captured eiders were similar to mean weights of molting birds captured at Izembek Lagoon in September (Flint, unpublished data) and Steller's Eiders wintering in Norway (Henriksen and Lund 1994 *in* Fox and Mitchell 1997*a*). Second, eiders lost about 1 gram of body weight per day between January and March in 2005 whereas Harlequin duck weights remained constant (Flint, unpublished data). Thus, it appears that wintering Steller's Eiders at Dutch Harbor are not forage limited and that they can meet most of their daily energy requirements in late winter with minimal time spent foraging. Previous studies found that wintering Steller's Eiders selected high energy prey species such as amphipods and gastropods (Bustnes *et al.* 2000, Bustnes and Systad. 2001*a*.). Thus, we hypothesize that relatively high densities of these invertebrates are available in the Dutch Harbor area.

If forage is readily available, why then do wintering sea ducks in the Dutch Harbor area show such wide movements? We suspect much of this movement is associated with predator avoidance. Large numbers of Bald Eagles (*Haliaeetus leucocephalus*) winter in the vicinity of Dutch Harbor. In previous years these eagles were observed to feed heavily on landfill wastes. Recent changes to landfill practices have altered forage availability for eagles and numerous eagles now roost along the sea cliffs. During our study, eagles were regularly seen attempting to take sea ducks in nearshore habitats. Thus, we suspect that optimal foraging habitats also have a relatively high risk of predation. Thus, eiders and Harlequin Ducks move into near-shore habitats to forage near sea-food processing and waste water effluents where invertebrate abundances are enhanced. But they quickly leave these areas once daily energy requirements are met to avoid high predation risks. Similarily, Fox and Mitchell (1997*b*) reported that Steller's Eiders wintering in Norway abandoned feeding areas in response to harassment from avian predators. This behavior of shifting between feeding and protected roosting sites may result in larger than normal home range size for birds wintering in the vicinity of Dutch Harbor.

Effects of point source impacts.

In the Dutch Harbor area there is particular interest in estimating the impact of specific

industrial developments and potential point sources of pollution. Our data indicate that the total number of birds influenced by a specific development would exceed the number of birds counted in the area at any one time as individuals are regularly moving into and out of most areas. Of particular interest during this study was use of the outfall area where both seafood processing plants and the municipal sewage plant maintain discharges. We observed that a few times per day the outfall area was visited for short periods (<1 hr) by large numbers of sea ducks. All but four of the radio-marked Steller's eiders and two-thirds of the Harlequin ducks were detected around the outfall area on multiple occasions. Due to the short duration of the visits, they are likely under-represented in our triangulation data. However, examination of home ranges shows that at least three-fourths of the eiders and one-half of the Harlequin ducks have home ranges that overlap the outfall area. We suspect that birds may be attached to these outfall areas by enhanced feeding opportunities associated with eutrophication. It may be positive effects of this eutrophication on invertebrate abundance that allows wintering sea ducks in the area to meet their daily energy requirements with minimal time spent foraging.

If our hypothesis regarding eagle harassment influencing patterns of habitat use is valid, then changes in management actions unassociated with marine systems may also influence patterns of habitat use and foraging. The concentration of eagles wintering in the vicinity of Dutch Harbor likely exceeds that which could be supported in the absence of artificial food. Actions taken to minimize eagle access to garbage may have had subsequent effects on sea duck foraging behavior, patterns of habitat use and survival.

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Table 1. Telemetry effort and detection success from locations around Dutch Harbor, Alaska, February and March 2004.

	Telemetry Search Area				
	Airport Beach[1]	Sewage Outfall	Inner Harbor	Little South America	South Captain's Bay
# Telemetry Scans	246	80	38	167	111
# Detections					
Steller's eider	2485	511	221	1946	678
Harlequin duck	2794	585	266	1273	894
# Definite Locations					
Steller's eider	889	189	58	841	292
Harlequin duck	869	100	83	330	553
# Triangulation Scans	90	35	-	78	75
# Triangulation fixes					
Steller's eider	170	3	-	225	68
Harlequin duck	260	6	-	83	142

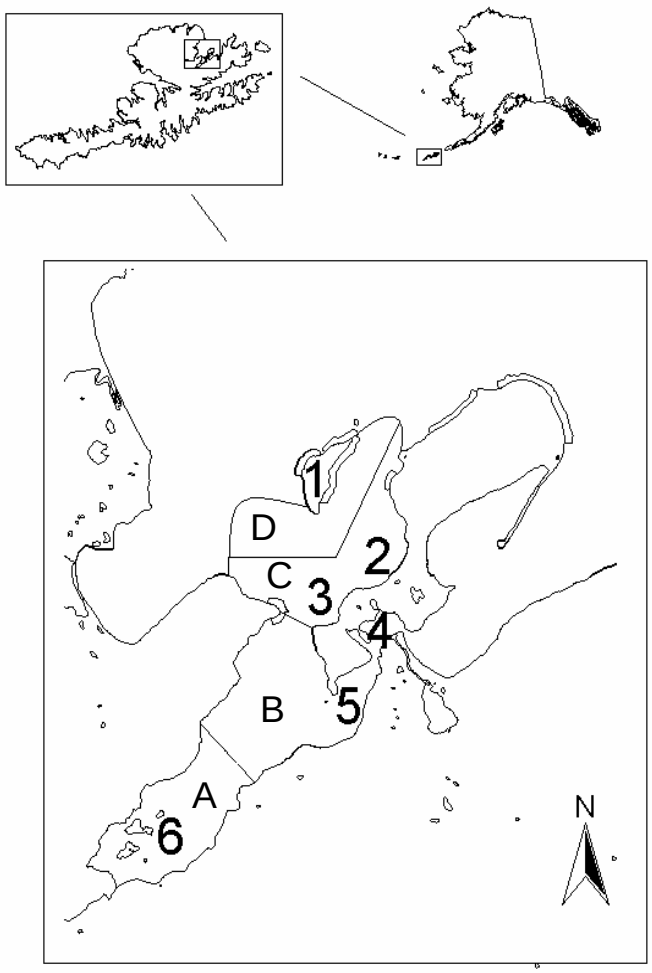
[1] includes birds near Hog Island

Table 2*a*. Daily probability of Steller's eiders moving among zones within the study area at Dutch Harbor, Alaska.

	TO			
From	Hog Island	Airport Beach	Little S. America	Captain's Bay
Hog Island		0.92	0	0.08
Airport Beach	0.02		0.06	0.02
Little S. America	0	0.07		0
Captain's Bay	0	0.31	0.21	

Table 2*b*. Daily probability of Harlequin ducks moving among zones within the study area at Dutch Harbor, Alaska.

	TO			
From	Hog Island	Airport Beach	Little S. America	Captain's Bay
Hog Island		0.65	0	0
Airport Beach	0.12		0.07	0
Little S. America	0	0.19		0.04
Captain's Bay	0	0.01	0.03	



Capture Locations
1. Hog Island
2. Airport Beach
3. Sewage Outfall
4. Inner harbor
5. Little South America
6. Captain's Bay

Telemetry Zones
A. Captain's Bay
B. Little South America
C. Airport Beach
D. Hog Island

Figure 1. Study area map identifying capture locations and telemetry zones in the vicinity of Dutch Harbor, Alaska.

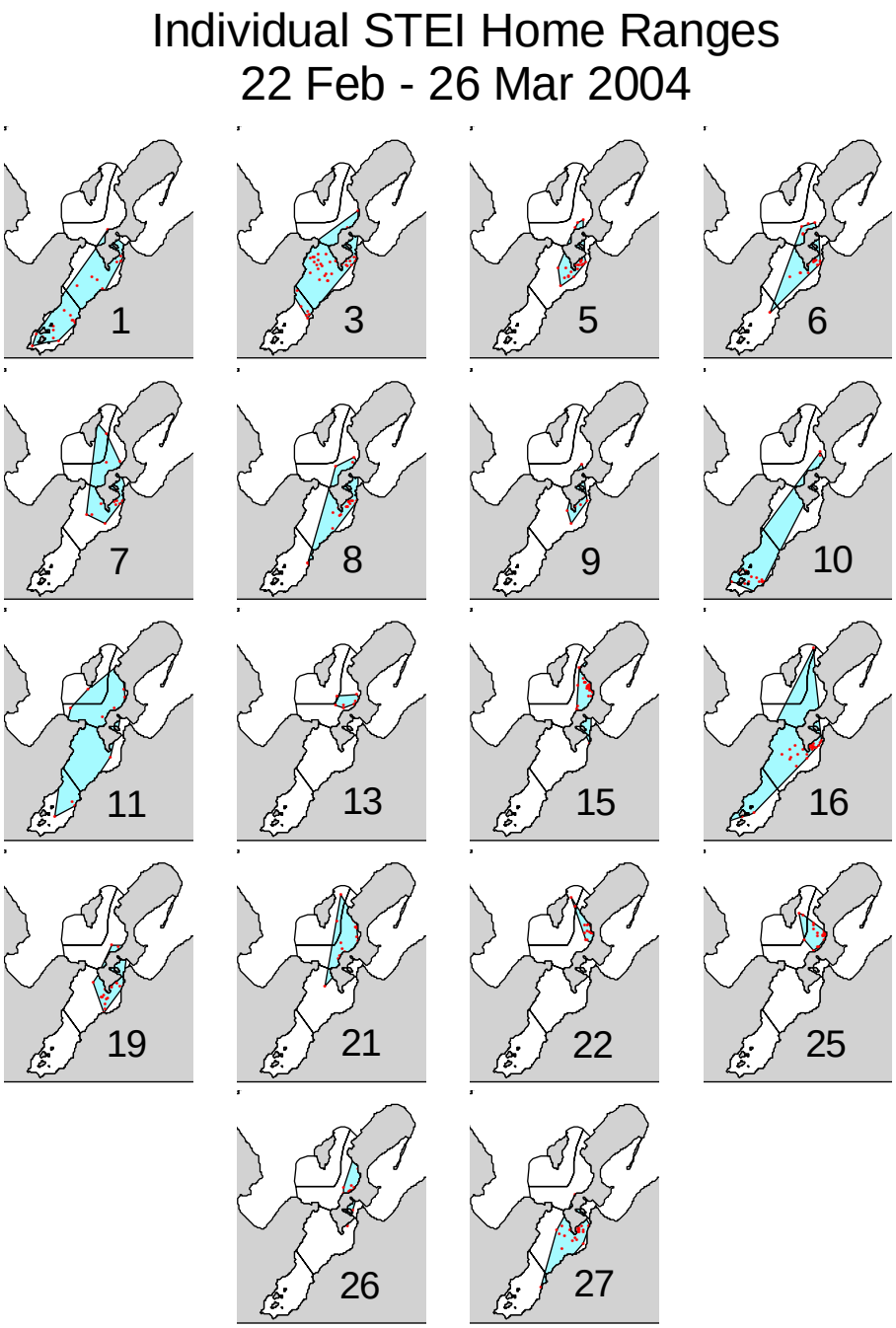


Figure 2*a*. Individual Steller's Eider home ranges (Minimum Convex Polygons), Dutch Harbor, Alaska 22 February – 26 March 2004. Blue shading represents MCPs and lines delineate the five zones within the study area.

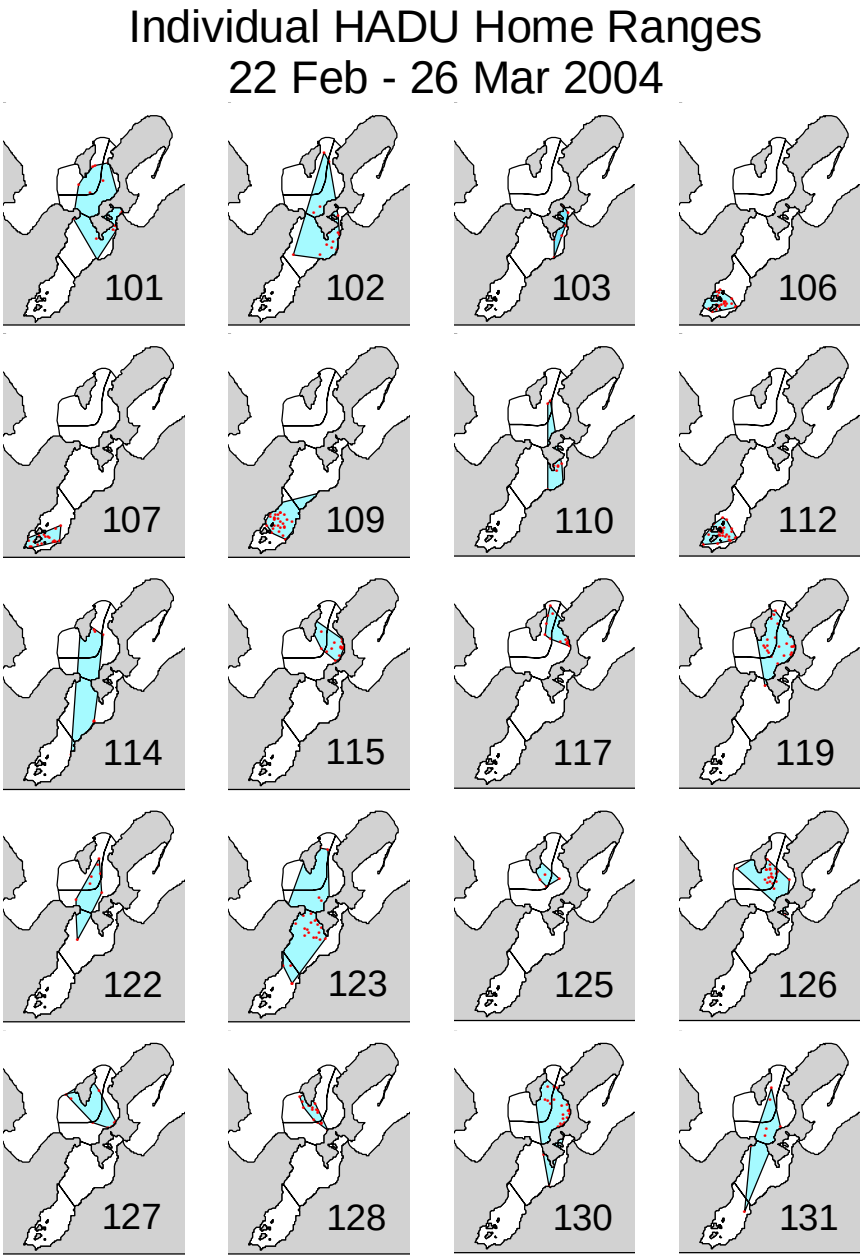


Figure 2*b*. Individual Harlequin duck home ranges (Minimum Convex Polygons), Dutch Harbor, Alaska 22 February – 26 March 2004. Blue shading represents MCPs and lines delineate the five zones within the study area.

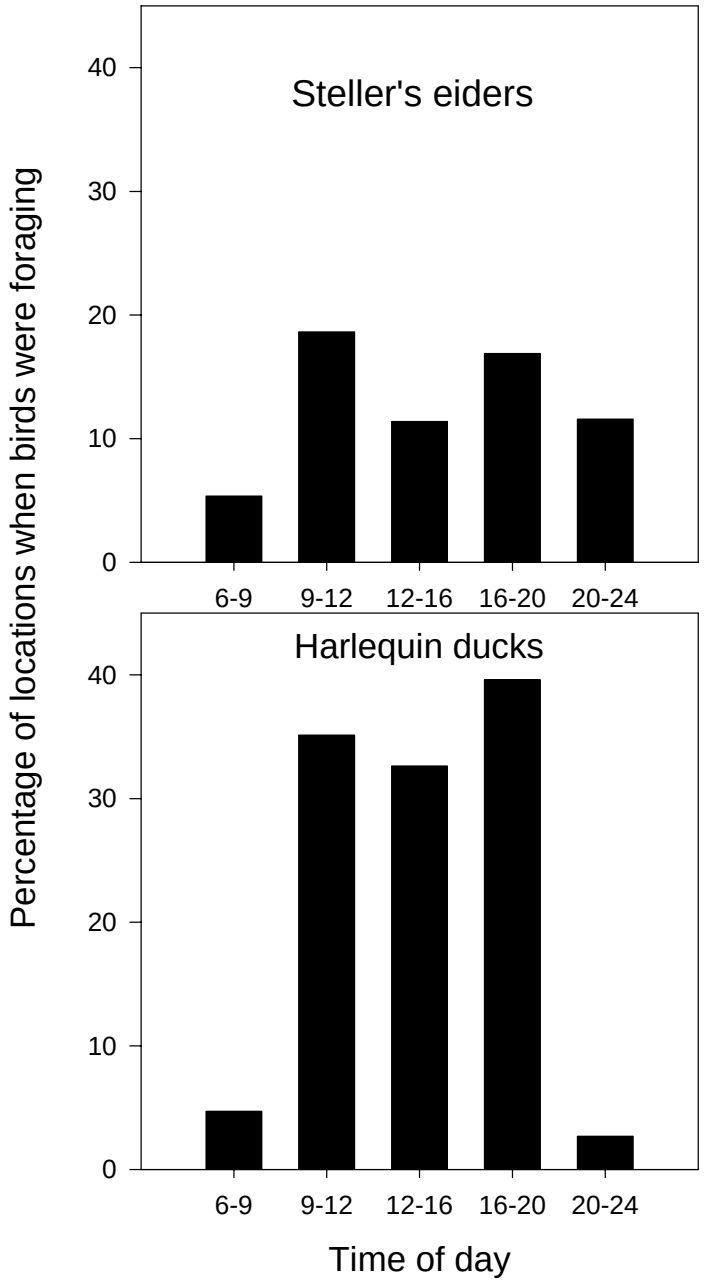


Figure 3. Index of time spent foraging for Steller's Eiders and Harlequin Ducks wintering near Dutch Harbor, Alaska from 22 February through 26 March 2004. For each observation of a radio transmitter, diving behavior was noted. Individual birds were pooled within time blocks and the proportion of time diving was calculated.

APPENDIX B:

Standard Operating Procedure for Surgical Biopsy of Seaduck Livers

Standard Operating Procedure for Surgical Biopsy of Seaduck Liver

SCOPE: This protocol applies to the surgical procedures required to obtain liver biopsies from waterfowl and sea birds.

PRINCIPLE: Procedures established for obtaining liver biopsies from pet birds are cumbersome and are not suited for field use on wild birds. The following procedure has been tested for use in the field, and is intended to produce a sample large enough (about 0.5 g) to allow analysis of cytochrome oxidases. When such a large sample is not needed, consideration should be given for obtaining a sample by the use of a Tru-Cut® biopsy instrument or by a fine needle aspirate.

PROCEDURES:

1. Two people are necessary to obtain the sample: a surgeon and an anesthetist. Because of the lability of cytochrome oxidases, a third person is required for the immediate handling of the sample.
2. Standard aseptic surgical technique will be practiced. The surgeon will wear sterile gloves and a surgical mask. The surgical site will be prepared as for any surgical procedure, including plucking feathers, skin disinfection (using povidone iodine or chlorhexidine) and the use of a sterile drape.
3. Surgical instruments should be sterilized in an autoclave with a minimum of two layers of packaging and stored in a dry place.
4. Position the bird on the surgical table in dorsal recumbancy with the legs extended and the wings folded. An insulated (bubble-wrap or foam pad) cover for the surgical table should be used to retard heat loss. Because regurgitation of crop contents is a frequent problem, birds should be intubated. Birds should be placed on an elevated platform with a sloped ramp, positioning the bird's head on the ramp so that it is lower than the body.
5. A single intramuscular dose of ketoprofen at 4 mg/kg is given during or immediately following induction for presumptive post-operative analgesia.
6. Isoflurane gas anesthetic is administered to the bird by facemask on a non-rebreathing circuit. Induction is at 4-5% isoflurane; maintenance is at 1-2% isoflurane in oxygen or at a level found necessary for a given species and a given individual. Maintenance concentrations of isoflurane may vary depending on the individual bird and environmental variables. The bird is intubated with a cuffless tube or with a cuffed tube without inflating the cuff. A protective ointment may be used in the eyes to prevent drying of the cornea. Once the abdominal air sacs of a bird are opened, respiration can occur partially through the surgical incision, which may require a higher setting on the vaporizer to compensate. Once the incision is closed, the vaporizer setting may need to be reduced. If oxygen is not available, compressed air (optimum: >50 psi; minimum: 30 psi) can be used to drive the vaporizer.
7. An alternative protocol is the intravenous administration of propofol in combination with a local anesthetic block at the incision site (Machin and Caulkett 1998, 2000). Propofol does not contain a preservative and supports bacterial growth, so every effort must be made to maintain the stock vial in a sterile state. Only new needles may be placed into a vial of propofol. Opened vials of propofol should be kept refrigerated if possible. Open vials should be discarded if asceptic technique is broken, or within 24 hours of being

opened. A 25 gauge 3/8 in. butterfly catheter is placed into the tibiotarsal vein (alternatively, a 21 gauge, 1 in. butterfly catheter may be placed into the jugular vein. The catheter is taped in place. Induction of anesthesia is accomplished by delivering a slow bolus (over 1 min) of 10 mg/kg propofol. Additional boluses of 1-2 mg may be given to attain induction and to maintain a surgical plane of anesthesia. All birds must be intubated, and ventilated with a bird AMBU bag. The incision site and the antenna exit site are infiltrated with 2 mg/kg of a 0.5% solution of bupivicaine, or of lidocaine.

8. Anesthesia is monitored by use of a respiratory or cardiac monitor, or both. A Doppler ultrasound the preferred monitor. An ECG is highly recommended. Manual palpation of a tibial or brachial arterial pulse can also be used, but is less preferred to an attached continuous mechanical monitor.
9. Body temperature is monitored with an electronic thermometer with the sensor placed either well into the esophagus or in the cloaca. The desired temperature range during anesthesia and surgery is 100º to 105º F. The bird should be warmed or cooled to maintain this range. Additional heat can be supplied to a cold bird by placing bags of warm water on the ventral surfaces of the wings or, ideally, by the use of a radiant heat source located above the bird. Body temperature can be reduced by removal of external heat sources and by wiping the feet with alcohol or cold water.
10. Respiration is monitored and mechanically supported when spontaneous breathing is less than one breath per minute. Two ventilations per min are made with a bird AMBU bag.
11. The surgical site is between the distal end of the keel and the conjuncture of the distal ends of the pubic bones, palpated through the abdominal wall. The feathers are plucked from the site. An area 1 cm on either side of the incision site should be plucked free of feathers. The feathers around the site are taped back with pieces of microporous tape. The site is swabbed twice with povidone-iodine or benzalkonium chloride solution. A sterile fenestrated drape is placed over the surgical site.
12. The skin is incised along the ventral midline with a No. 11 or No. 15 sterile blade. The subcutaneous layer and fat are sharp dissected. Once the muscular abdominal wall is reached, the linea alba is identified. The linea alba is seized with a forceps and lifted to permit penetration of the abdominal wall with a blade. The linea alba is then sharp dissected with blade or scissors, avoiding the viscera, to a length of about 2 cm, or a distance sufficient to pass the transmitter body.
13. The caudal edge of the liver is located in the abdomen. Occasionally the liver is located too far rostrally to permit easy access. In those cases, the surgeon should pick up the bird, keeping his hands on the sterile side of the drape, elevate the bird's head, and shake the bird gently. The liver should drop down into view.
14. A piece of the caudal edge of the liver is isolated using a curved mosquito forceps. The liver should fill the entire arc of the forceps. The jaws of the forceps are closed, but not locked, crushing the tissue. The isolated piece of liver is then cut free by passing a scalpel blade along the inside curve of the forceps. The cut piece of liver is then placed on a sterile sponge and dropped onto the surgical table outside the sterile field, for the sample processor to pick up.
15. The surgeon maintains the crush of the cut edge of the liver for about 10 seconds. Then the forceps is gently released, observing for hemorrhage. If bleeding is observed, the forceps are gently applied to the bleeding part and pressure is held for another 10 sec. The forceps are then gently removed and the cut edge observed for bleeding. Gel foam can be

applied to the cut edge of the liver if continued bleeding is a problem.

16. The surgical incision is closed in two layers using 3-0 braided absorbable sutures on a cutting needle. The linea alba is closed using a simple continuous pattern and the skin is closed using either a simple continuous or simple interrupted pattern.
17. The drape is removed and the vaporizer is turned to zero. Oxygen supplementation should continue until the bird recovers. Additional procedures such as obtaining a blood sample or banding may be done during this period. The bird should be kept warm by holding it wrapped in a towel until it is fully recovered. If dehydration is a problem, subcutaneous fluids can be administered.
18. Following recovery, the bird should be placed in a cage or kennel for at least one hour prior to release.

REFERENCES:

Machin, K. L., and N. A. Caulkett. 1998. Investigation of injectable anesthetic agents in mallard ducks (Anas platyrhynchos): a descriptive study. J. Avian Med. Surg. 12 (4): 255-262.

Machin, K. L., and N. A. Caulkett. 2000. Evaluation of isoflurane and propofol anesthesia for intraabdominal transmitter placement in nesting female canvasback ducks. J. Wildl. Dis. 36(2):324-334.

APPENDIX C:

Nutritional content for Sea Duck Diet

Nutritional content for Sea Duck Diet (©1996-2004 PMI Nutrition International, Purina Mills, Inc.) and raw blue muscle NDB # 15164 or raw snail, NDB # 90560 (USDA National Nutrient Database for Standard Reference 2004)

	Mazuri Sea Duck Diet	Blue Muscle	Snail
Energy (kcal/g)	3	1	1
Proximate Analysis			
Water %	0	80.6	79.2
Protein %	21.6	11.9	16.1
Total lipid (fat) %	6.5	2.2	1.4
Ash %	10.9	1.6	1.3
Carbohydrate %	44.6	3.7	2.0
Fiber, total dietary %	8.4	0	0
Other %	8.0	0	0
Minerals			
Sodium %	0.37	0.28	0.07
Phosphorous %	1.3	0.20	0.27